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DIETARY INTAKES

Dietary patterns, beliefs and behaviours among individuals with inflammatory bowel disease: a cross-sectional study

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Abstract

Background: Inflammatory bowel disease (IBD) refers to a group of incurable gastrointestinal diseases that are common among young adults. The present study aimed to describe dietary intake, self-modifications and beliefs among adults aged 18–35 years with IBD and to compare those with active versus inactive disease. National guidelines for daily intake include: 34 g of fibre for males and 28 g of fibre for females, 3 oz of whole grains, 1000 mg of calcium, <10% of added sugars, three cups of dairy, 2.5 cups of vegetables and two cups of fruit.
Methods: Individuals with a diagnosis of IBD were recruited online using convenience sampling (n = 147). Data included a dietary screening questionnaire, self-directed diet modifications, dietary beliefs questionnaire and demographics. Chi-squared and t-tests were used to compare those with active versus inactive disease.
Results: The sample was predominantly female (90%) and diagnosed with Crohn's disease (64%). Daily intake for females was 9.7 g of fibre, 0.3 oz of whole grains, 683.8 g of calcium, 1.1 of cups vegetables and 0.5 of cups fruit. Daily intake for males was 14.2 g of fibre, 0.4 oz of whole grains, 882.9 g of calcium, 1.4 cups of vegetables and 0.5 cups of fruit. Participants most often modified fibre (73%), fruits and vegetables (71%), grains (67%), and dairy (66%) as a result of their IBD. Eighty-three percent believed that modifying their diet could reduce IBD symptoms.
Conclusions: Both men and women with IBD struggle to meet the national guidelines for intake of fibre, whole grains, fruits and vegetables. The majority reported modifying their dietary intake as a result of IBD and expressed belief that diet could reduce symptoms.

Introduction

Inflammatory bowel disease (IBD) refers to a group of chronic inflammatory diseases of the gastrointestinal system. IBD is a heterogeneous disease that is typically classified into ulcerative colitis and Crohn's disease. The disease course varies, with individuals experiencing periods of active disease and remission. IBD is a growing public health concern because the incidence has been increasing worldwide [(1)]. IBD is associated with gastrointestinal symptoms (e.g. abdominal cramps, abdominal pain and diarrhoea) and inflammation of the gastrointestinal tract [(2,3)]. Because IBD affects the gastrointestinal system, patients often question how modifying their diet may influence IBD symptoms [(4–6)]. Some studies have shown the benefits of diet modification, through altering food intake, on symptom reduction and quality of life in IBD patients [(5,7–9)]. Indeed, children with Crohn's disease on 8 weeks of exclusive enteral nutrition, a nutritionally complete liquid diet, demonstrated a greater likelihood of remission compared to children who do not use exclusive enteral nutrition [(10)]. Although studies demonstrate the

effectiveness of dietary interventions [i.e. specific carbohydrate diet or low fermentable oligo-, di-, mono-saccharides and polyols (FODMAP) diet] in reducing symptoms, few studies have demonstrated changes in markers of inflammation [11–16]. Although dietary recommendations have been published for adults with IBD, recommendations primarily report insufficient evidence to recommend dietary changes and are based on expert opinions [17,18]. Therefore, providers have been hesitant to provide specific recommendations as a result of the complex role of diet in IBD and the lack of a dietary gold standard [5,19].

Even without clear dietary recommendations, individuals with IBD anecdotally report diet modifications. Adults with IBD are interested in diet because patients believe that modifying their diet could decrease IBD symptoms and improve health [20–23]. Yet, dietary information is reported as the least adequately addressed topic among individuals who have been recently diagnosed and those with longstanding disease (>10 years) [24,25]. Furthermore, even though diet modification is commonly reported in clinical settings, few studies have characterised dietary intake (i.e. grams of fibre, cups of fruits and vegetables) among adults with IBD [26]. Characterisation of dietary intake is the first step towards better understanding nutrient deficiencies and developing personalised dietary recommendations for patients.

The present study describes the current dietary intake, modifications and beliefs among adults aged 18–35 years with IBD and compares dietary modifications between individuals with active and inactive disease. Dietary research among individuals with IBD is limited and primarily focuses on patients beliefs about specific types of food [20,22,23]. Because dietary modification is common, there is a need to describe the current dietary intake and modifications among individuals with IBD in the USA. Dietary intake will be compared with the USA Department of Agriculture (USDA) recommendations. These recommendations propose 34 g of fibre for males and 28 g of fibre for females, 3 oz of whole grains, 1000 mg of calcium, <10% of added sugars, three cups of dairy, 2.5 cups of vegetables and two cups of fruit [27].

The present study addresses three research questions:

- Among individuals with IBD, does dietary intake (fibre, whole grains, calcium, sugar, dairy, vegetables and fruit) differ between those with active and inactive disease?
- Are individuals with active IBD more likely to modify their diet than individuals with inactive IBD?
- Do dietary beliefs differ between individuals with active and inactive IBD?

Materials and methods

Study design

A cross-sectional study design was conducted to describe the current dietary intake, modifications, and beliefs among adults aged 18–35 years with IBD. The study was approved by the Michigan State University Institutional Review Board.

Participants

Individuals were recruited through ResearchMatch, Facebook or referral from a friend between January and February 2018. Individuals were screened for participation if they had a diagnosis of ulcerative colitis or Crohn's disease and were aged 18–35 years. Inclusion criteria were: currently prescribed medication to manage their IBD, living in the USA, an understanding of written English and access to the Internet. Those hospitalised within the past month or currently pregnant were excluded.

Procedures

All data were collected using Qualtrics. Those interested in participating reviewed an online consent form describing the purpose of the study, study procedures and voluntary participation. Participants first completed required screening questions to assess for eligibility. These questions were automatically scored, and those meeting inclusion/exclusion criteria continued to the survey.

Measures

Participant characteristics

Participants were asked about their age, gender, and marital status. Participants reported type of IBD (ulcerative colitis or Crohn's disease), time since diagnosis and current medication type (aminosalicylates, biologics, corticosteroids and/or immunomodulators). Disease activity was assessed using the Manitoba Inflammatory Bowel Disease Index (MIBDI), where participants report their disease activity on a six-point scale ranging from 'constantly active, giving me symptoms every day' or 'I was well in the past 6 months, what I consider a remission or absence of disease'. A cut-off of 1–4 for active disease and 5–6 for inactive disease was used based on the existing literature [28].

Dietary intake

Dietary Intake was assessed by the 26-item National Health and Nutrition Examination Survey (NHANES) and National Cancer Institute dietary screener

questionnaire. Participants reported the frequency of food and drink consumption over the past month as the number of times per day, week or month. Estimates for intake of food and nutrient groups were calculated using publicly available scoring algorithms [29]. The algorithms were developed based on 24-h dietary recalls [30] and account for participant age and sex. Food and nutrient groups include per day measures of: fibre (g), calcium (mg), whole grains (ounce equivalents), total added sugars (tsp equivalents), dairy (cup equivalents), fruits and vegetables (cup equivalents) and added sugars from sugar-sweetened beverages (tsp equivalents).

Dietary modification

After each item on the Dietary Screener Questionnaire [7,30], participants responded to the following statement: 'I alter my intake of [name of food] due to my IBD' on a Likert scale (0 = never to 5 = always). Responses were then categorised into subscales (fruit and vegetables, dairy, whole grains, added sugars, sugar-sweetened beverages, meat, and dietary fibre) based on the scoring algorithms. In addition, the most frequently modified individual foods were reported. Participants who reported never or rarely were categorised as no diet modification; participants responding sometimes, often or always were categorised as modifying their diet.

Dietary beliefs

Dietary beliefs were assessed using investigator-developed questions, which were pretested prior to survey use. Participants were asked questions such as 'Do you think that diet modification can reduce IBD symptoms?' and 'Does your healthcare provider think that diet modification can reduce IBD symptoms?' Participants could respond 'yes', 'no' or 'don't know'. In addition, participants were asked if they could identify foods that made their symptoms better or worse and if they had ever seen or were currently seeing a dietitian/nutritionist for their IBD.

Statistical analysis

Data were analysed using STATA, version 15.1 (StataCorp, College Station, TX, USA) and SAS, version 14.1 (SAS Institute Inc., Cary, NC, USA). Demographic statistics were calculated using the means (SD) for continuous variables, and counts and percentages for categorical variables. Dietary intake was calculated in SAS using scoring procedures published by the National Cancer Institute [29]. Data were reported as the means (SD). Comparisons were made between individuals with active and inactive disease using *t*-tests.

Dietary modification was scored as a dichotomous variable and presented as the percentage of individuals modifying or not modifying their diet. Chi-squared was used to assess the relationship between diet modification and disease activity. Dietary beliefs were presented as counts and percentages.

Results

Participant characteristics

One-hundred and forty-seven individuals met the inclusion criteria. The sample was predominantly female (90%), with a mean (SD) age of 28.8 (4.6) years, and diagnosed with Crohn's disease (64%). The majority of participants were on a biological medication (64.6%), and 38.1% took more than one medication type. Based on the MIBDI scale, 75% of the sample reported active disease (Table 1).

Dietary intake

Dietary intake was reported for individuals who did not have missing data on the individual scale items. The estimated dietary intake for males (n = 12) each day was 14.2 g of fibre, 0.4 oz of whole grains, 882.9 g of calcium, 1.4 cups of vegetables and 0.5 cups of fruit. Estimated dietary intake for females (n = 113) each day was 9.7 g of fibre, 0.3 oz of whole grains, 683.8 g of calcium, 1.1 cups of vegetables and 0.5 cups of fruit. Females with active compared to inactive disease differed on predicted intake of fibre (P = 0.006), calcium (P = 0.013), and fruit and vegetable intake (all <0.01). For additional dietary intake information, see Table 2.

Table 1 Demographics of individuals with inflammatory bowel disease (IBD), aged 18–35 years

	Mean	SD
Age	28.8	4.6
Time since diagnosis (in years)	7.6	5.6
	N	%
Female sex	132	89.8
Single	79	53.7
Type of IBD		
Ulcerative colitis	53	36
Crohn's disease	94	64
Medication type		
Aminosalicylates	51	34.5
Biologics	95	64.6
Corticosteroids	22	15.0
Immunomodulators	33	22.5
Manitoba IBD index		
Active disease	110	74.8
Inactive disease	37	25.2

Some individuals were using multiple medications.

Table 2 Predicted dietary intake for males and females with inflammatory bowel disease

				Females		
	USDA Recommendations	Males (n = 12) Mean (SD)	Females (n = 113) Mean (SD)	Active disease (n = 82) Mean (SD)	Inactive disease (n = 30) Mean (SD)	Active versus inactive disease P-value
Fibre (g)	34 males/28 females	18.2 (3.3)	14.3 (2.8)	13.9 (2.4)	15.5 (3.3)	0.006
Predicted intake of calcium (mg)	1000	1129.3 (257.9)	853.3 (104.6)	838.8 (88.1)	894.5 (134.8)	0.013
Whole grains (ounce equivalents)	3	0.92 (0.5)	0.6 (0.3)	0.6 (0.3)	0.7 (0.4)	0.16
Total added sugars (tsp equivalents)	<10%	15.8 (2.5)	15.9 (5.3)	16.2 (5.7)	15.0 (4.1)	0.29
Dairy (cup equivalents)	3	1.8 (0.7)	1.4 (0.4)	1.4 (0.3)	1.5 (0.5)	0.20
Fruits and vegetables including legumes and French fries (cup equivalents)	4.5	2.7 (0.7)	2.2 (0.5)	2.1 (0.4)	2.4 (0.6)	0.003
Vegetables including legumes and French fries (cup equivalents)	2.5	1.7 (0.5)	1.4 (0.2)	1.2 (0.2)	1.5 (0.3)	0.0001
Fruits and vegetables including legumes and excluding French fries (cup equivalents)	4.5	2.5 (0.7)	2.1 (0.5)	2.0 (0.5)	2.3 (0.6)	0.01
Vegetables including legumes and excluding French fries (cup equivalents)	2.5	1.6 (0.5)	1.2 (0.3)	1.2 (0.2)	1.4 (0.3)	0.0001
Fruits (cup equivalents)	2	0.9 (0.4)	0.8 (0.3)	0.8 (0.3)	0.9 (0.3)	0.21
Added sugars from sugar-sweetened beverages (tsp equivalents)	<10%	0.2 (0.1)	7.1 (4.7)	7.5 (4.9)	6.2 (3.8)	0.19

All data are presented as the predicted intake, per day; dietary analysis was performed on individuals with complete dietary intake responses.

Dietary modifications

Dietary modification was common within the sample with the majority of individuals (83%) modifying their diet due to IBD (Table 3). Participants most commonly reported modifying intake of fibre (73%), fruits and vegetables (71%), grains (67%) and dairy (66%). Dietary modification was greater among individuals with active disease compared to inactive disease (all $P < 0.05$). The individual food items most frequently modified included: salad (72%), popcorn (72%), other vegetables (67%), fruit (66%) and pizza (62%).

Dietary beliefs

Sixty-nine percent of participants reported that diet modification could reduce IBD symptoms (Table 4). Only 47.6% of participants reported their healthcare provider thought diet modification could reduce IBD symptoms. Twenty-five percent reported ever visiting a dietitian and/or nutritionist for their IBD and only 5% were currently seeing a dietitian and/or nutritionist. There were no differences in dietary beliefs based on disease activity.

Discussion

Among an online sample of primarily females aged 18–35 years with IBD, food intakes based on a dietary screener questionnaire did not meet USDA recommendations [27]. Participants reported commonly modifying foods as a result of their IBD, with the most commonly modified foods being fibre, fruits and vegetables, and grains. Individuals were more likely to modify their diet during active disease.

Individuals with IBD often do not obtain the necessary nutrients. For instance, the USDA recommended an intake of 34 g of fibre for males and 28 g of fibre for females per day [27]. Participants in the present study had a predicted fibre intake of 18 g for males and 14 g for females, which is similar to actual fibre intake in the general U.S. population (17 g) [31,32]. A Canadian study found significant micronutrients deficits (vitamins C, D, thiamin, and niacin) among individuals with Crohn's disease compared to a representative sample of Canadians [26]. Increased dietary fibre intake was found in patients with Crohn's disease compared to the representative sample of Canadians [26], whereas an Italian cohort found decreased dietary fibre, increased lipids and increased calories among IBD patients compared to controls [33]. Differences in fibre intake may be a result of the percentage of individuals following a low-residue or low-fibre diet. Such variations demonstrate a need to characterise the current dietary intake of individuals with IBD prior to providing dietary recommendations.

Dietary modification was common among the sample, with individuals in active disease more likely to modify

Table 3 Dietary modifications among individuals with inflammatory bowel disease based on food subgroups

	Total sample		Active disease		Inactive disease		
	n	%	*n*	%	*n*	%	*P*-value
Modify fibre							
Yes	108	73.5	86	78.2	22	59.5	0.026
No	39	26.5	24	21.8	15	40.5	
Modify fruits and vegetables							
Yes	105	71.4	85	77.3	20	54.1	0.007
No	42	28.6	25	22.7	17	46.0	
Modify grains							
Yes	99	67.4	80	72.7	19	51.4	0.016
No	48	32.7	30	27.3	18	48.7	
Modify dairy							
Yes	97	66.0	79	71.8	18	48.7	0.01
No	50	34.0	31	28.2	19	51.4	
Modify added sugar							
Yes	89	60.5	76	69.1	13	35.1	0.001
No	58	39.5	34	31.0	24	64.9	
Modify sugar beverages							
Yes	78	53.1	66	60.0	12	32.4	0.004
No	69	46.9	44	40.0	25	67.6	
Modify meat							
Yes	77	52.4	65	59.1	12	32.4	0.005
No	70	47.6	45	40.9	25	67.6	

P-value comparing active versus inactive disease.

Table 4 Beliefs about diet among individuals with inflammatory bowel disease (IBD)

	Total sample		Active disease		Inactive disease		
	n	%	*n*	%	*N*	%	*P*-value
Have you seen a dietitian and/or nutritionist for your IBD?							
Yes	36	24.5	27	24.6	9	24.3	0.72
No	109	74.2	82	74.2	27	73.0	
Are you currently seeing a dietitian and/or nutritionist for your IBD?							
Yes	9	6.2	7	6.4	2	5.4	0.98
No	136	93.8	103	93.6	35	94.6	
Do you think that diet modification can reduce IBD symptoms?							
Yes	102	69.4	78	70.9	24	64.9	0.6
No	21	14.3	16	14.6	5	13.5	
Did not respond	24	16.3	16	14.6	8	21.6	
Does your healthcare provider think that diet modification can reduce IBD symptoms?							
Yes	70	47.6	53	48.2	17	46.0	0.6
No	39	26.5	27	24.6	12	32.4	
Did not respond	38	25.9	30	27.3	8	21.6	
Are you able to identify foods that make your IBD worse?							
Yes	124	87.3	94	89.5	30	81.1	0.18
No	18	12.7	11	10.5	7	18.9	
Are you able to identify foods that make your IBD better?							
Yes	55	41.0	38	37.6	17	51.5	0.16
No	79	59.0	63	62.4	16	48.5	

P-value comparing active versus inactive disease.

their diet than individuals in inactive disease. Yet, during inactive disease, one-third to two-thirds of participants still engaged in some type of dietary modification. A Dutch study reported 76.5% of their sample of individuals with IBD omitted foods to reduce disease symptoms [34]. Specifically, participants felt that omitting foods reduced

abdominal pain/cramps and diarrhoea. Another study reported that 66.8% of IBD patients avoid certain food to prevent a relapse [23]. Individuals with IBD focus on eliminating foods that worsen symptoms and few individuals focus on incorporating foods that improve symptoms [35]. In the current sample, 41% of participants were able to identify foods that made their IBD better. This points to a possible need to reframe nutritional instructions.

Within this sample, 24.8% of individuals visited a dietitian or nutritionist and only 6% were currently receiving services from a dietitian or nutritionist. This is similar to findings within a Dutch cohort in which 25.3% of individuals obtained nutrition information from a dietitian [34]. Although few individuals have obtained guidance from a dietitian or nutritionist, the majority of participants in the current study reported modifying their diet. This is especially concerning because recent research in an Italian cohort demonstrated an association between self-prescribed dietary restrictions and abnormal bone density scans [36]. Furthermore, individuals with IBD are at risk for nutrient deficiencies as a result of bowel malabsorption [37]. Future research could target those with self-prescribed dietary modifications in the absence of a dietitian/nutritionist. Such modifications could have a social or emotional component, as well as physical discomfort. With advanced knowledge, more tailored interventions could be designed to address the rationale for self-prescribed changes. In the meantime, providers should encourage patients to enlist the help of a registered dietitian/nutritionist that specialists in nutritional therapy for IBD aiming to prevent nutritional deficits. A registered dietitian can help patients identify foods that affect their symptoms, as well as prevent and help with recovery from malnutrition, nutrient deficiencies and the fear of eating that often accompanies IBD.

The limitations of the present study include that the sample was recruited online using convenience sampling and may not be representative of individuals in clinical settings. Specifically, the small sample size of males limited understanding of the dietary intake specific to males. Future studies with a larger sample size can better characterise differences based on sex, disease activity and other factors that may influence diet. Although a validated dietary screener was used to predict food intake, the use of a 3-day dietary intake would provide more detailed information on both micro- and macronutrients.

Conclusions

The present study addressed the pressing clinical challenge of patient dietary modification by providing new information on current dietary intake, modifications and beliefs among individuals with IBD living in the USA. The findings indicated the majority of individuals are modifying their diet without the guidance of a dietitian or nutritionist. Participants had a lower dietary intake of fibre, whole grains, fruits and vegetables than recommendations. Larger studies would be beneficial with respect to assessing dietary intake for both men and women. If sex differences were found, tailored interventions could be designed and tested for their effectiveness in curbing symptoms and nutritional concerns. Longitudinal studies examining dietary intake over time may also provide additional insights into factors that influence dietary intake. This work provides the foundation for future work in the area of dietary intervention trials aiming to determine the best diet composition for individuals with IBD, and perhaps unique components for men versus women.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with STROBE guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

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Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.
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KK drafted the manuscript. KK, GW and BG designed the study. KK and BP analysed the data. KK, BP, DJ, GW and BG interpreted the data, provided substantial revisions, and reviewed the final manuscript. All authors critically reviewed the manuscript and approved the final version submitted for publication.

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DIETARY INTAKES

Exploring dietary changes in an interdisciplinary intervention trial: Application of a dietary guidelines food composition database

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Abstract

Background: Consumption of food groups aligning with dietary guidelines is advised for obesity management and was used in a recent lifestyle intervention trial, the Health Track study. We have conducted a number of dietary pattern analyses on this trial but, with recent access to the new Australian Dietary Guidelines (ADG) food composition database, we can now assess ADG adherence, with the advantage of categorising mixed dishes. The present study aimed to compare changes over time in consumption of ADG food groups.
Methods: Secondary analysis of baseline and three-month diet history data was conducted. Participants received individualised dietary advice (*I*), individualised dietary advice plus 30 g of walnuts per day (IW) or usual care (*C*). The ADG database was used to determine food group servings with changes in five food groups used as a measure of dietary quality.
Results: Fruit and vegetable intakes increased in the IW (+0.4 and +1.1 serves, $P < 0.05$) and *I* (+0.5 and +0.4 serves, $P > 0.05$) arms. Consumption of meat/protein foods increased in the IW arm (+0.3 serves, $P > 0.05$) but decreased in the *I* and *C* arms (both − 0.4 serves, $P < 0.05$). Consumption of grains and milk/alternatives decreased in all study arms ($P < 0.05$). Greater improvements in grain and dairy food quality were observed in the intervention arms.
Conclusions: The ADG database enabled ADG specific food group analysis, addressed food quality and showed the HealthTrack intervention increased adherence to dietary guidelines compared to usual care.

Introduction

Dietary guidelines provide an evidence-based synthesis that is used to inform nutrition policies, targets and health messages around the world. [1]. The Australian Dietary Guidelines (ADG) advise the consumption of dietary patterns to prevent chronic disease and optimise energy balance from five food groups [2]. Additionally, the guidelines advise limiting discretionary foods (those high in saturated fat, added sugars, added salt and alcohol). Research suggests greater adherence to dietary guidelines is associated with a reduced risk of lifestyle diseases, including obesity, type 2 diabetes, cardiovascular disease and certain cancers [3–8]. Measuring and promoting adherence to dietary guidelines are key strategies for addressing obesity [8]. Given the prevalence of obesity globally, exploring adherence to dietary guidelines is warranted.

Examining adherence to dietary guidelines in individuals and population groups requires quantification of food group serves from dietary intake data. Historically, this has been challenging for mixed dishes spanning multiple food groups (e.g. porridge contains ingredients belonging to the grains and dairy food groups). Although food

composition databases capable of breaking mixed dishes into individual food groups such as the MyPyramid Equivalents Database [9] are available internationally, differences in the food supply between countries necessitate country specific databases [10]. Food Standards Australia New Zealand created the ADG database for analysing Australian Health Survey (AHS) 2011–2012 dietary intake data. [11] These data have been compared with intake recommendations for five food groups found in the Australian Guide to Healthy Eating (AGHE) indicating that Australians are not complying with dietary guidance [11].

Although the ADG database has been used to explore population-based intakes of food groups, to our knowledge, there are no published studies that have applied the ADG database to data collected in the context of an intervention study. It was hypothesised that the application of the database would provide insights into the impact of AGHE recommendations on food choices [12]. Therefore, the present study aimed to use the ADG food composition database to compare between group changes in food consumption during the HealthTrack study.

Materials and methods

The HealthTrack Study was a 12-month randomised controlled trial that compared a novel interdisciplinary lifestyle approach to weight loss with usual care [13]. HealthTrack Study participants were overweight/obese volunteers living in the Illawarra/Shoalhaven region and were otherwise healthy. Exclusion criteria have been reported elsewhere [13]. Participants were randomised to one of three study arms: intervention (*I*), intervention +30 g of walnuts (IW) and control (*C*). The interdisciplinary intervention consisted of individualised dietary advice supported by the AGHE from an Accredited Practising Dietitian (APD), categorical exercise advice supported by an exercise physiologist and counselling from a health coach. The control group received general recommendations based on the AGHE and Australian physical activity guidelines. The 30 g of walnuts provided to the IW arm were included in dietary modelling [14] to preserve energy balance. Dietary data were collected at baseline, three, six, nine and 12 months using a diet history interview [15] To enable compatibility with the AUSNUT 2011–2013 coding of the ADG database, data were updated to the AUSNUT 2011–2013 database using a systematic process described elsewhere [16]. The HealthTrack Study was registered on the Australian New Zealand Clinical Trial Registry (www.anzctr.org.au, ANZCTRN 12614000581662).

The present study was a secondary analysis of HealthTrack Study diet history data at baseline and 3 months, the interval of greatest intervention intensity [13]. This study compares the number of ADG database food group serves consumed per day between the time points and study arms. The diet history method was selected for these analyses as a result of its ability to capture more detailed food data that address a usual period of intake.

The ADG database was used to convert the diet history data from grams of food to the number of food group serves at each time point (Fig. 1). For each AUSNUT 2011–2013 food item, the ADG database provided the number of serves of all constituent food groups per 100 g of the AUSNUT food item. Although intuitive for foods belonging to a single food group (e.g. 100 g banana provides 0.67 serves of fruit), the process was challenging for composite foods (e.g. 100 g of lasagne provides 0.70 serves of grains, 0.45 serves of vegetables, 0.26 serves of dairy/alternatives and 0.22 serves of meat/protein foods). Some discretionary foods also contributed to ADG database food group serves (e.g. certain cocktails contain the food group ingredient fruit), whereas others do not (e.g. soft drink contains no food group ingredients). The present study analysed food group intakes regardless of origin. Applying the ADG database to the HealthTrack Study data provided the total number of food group serves per day for each participant.

All data matching, manipulation and summarisation were undertaken using EXCEL (Microsoft Corp., Redmond, WA, USA). A four step process was required.

Average daily diet history data (Step 1) was matched to the ADG database (Step 2) using the unique food ID codes from AUSNUT 2011–2013. This provided the number of constituent food group serves (Step 3) of the foods consumed by each participant. The number of food group serves was then aggregated to obtain the total number of food group serves per day, per participant (Step 4). To determine each food group's relative contribution to total energy intakes, the number of food group serves per MJ of total energy was calculated. The recommended number of food group serves per day [17] was determined for each participant, and the proportion of participants meeting recommendations at each time point was calculated.

Statistical analysis

SPSS, version 21.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. Only participants with diet history data available at both baseline and 3 months were included in the analyses. As a result of the skew of the data, median and interquartile ranges of food group serves and food group serves per MJ at each time point were determined. The Wilcoxon signed rank test was used to test changes in the median numbers of food group serves and food group serves per MJ from baseline to 3 months within each study arm were significant. The

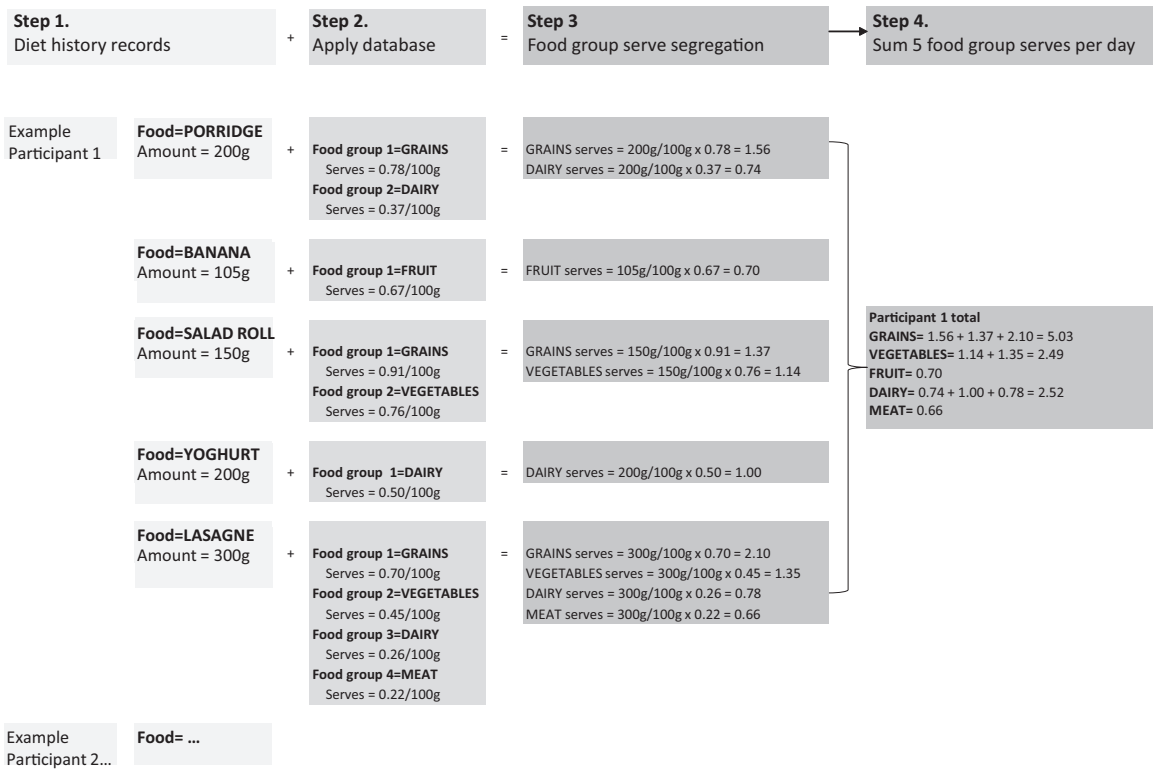

Figure 1 Process of applying the Australian Dietary Guidelines database to HealthTrack Study diet history data.

Kruskal–Wallis test with Bonferroni adjustment was used to explore differences in food group serves and per MJ between study arms at each time point. Changes in the proportion of participants meeting ADG recommendations from baseline to 3 months within each study arm were tested using McNemar's test and were considered to be a measure of change in dietary quality in this study. The difference in the proportion of participants meeting ADG recommendations between study arms at each time point was explored using the chi-squared test. $P < 0.05$ was considered statistically significant.

Ethical approval

Approval was granted by the University of Wollongong/Illawarra Shoalhaven Local Health District Human Research Ethics Committee (HE 13/189).

Results

In total, 293 participants had diet history data available at both baseline and 3 months. Baseline characteristics of the participants are shown in the Supporting information (Figure S1 and Table S1).

There was a reduction in the consumption of grains by over one serve in all study arms over the 3 months ($P = 0.000$) (Table 1). Despite this, intakes of wholegrains increased in all arms, although this was significant in the *I* arm only ($P = 0.001$). Refined grain intakes fell in all arms ($P = 0.000$) and intakes were significantly lower in the IW arm than the control arm at 3 months ($P = 0.001$). Vegetable intakes were significantly higher in the control arm than the IW arm at baseline ($P = 0.016$). Consumption of vegetables increased by 1.1 serves in the IW arm over the 3 months ($P = 0.000$). Within the vegetable food group, intakes of green/brassica vegetables rose by 0.4 serves in the IW arm ($P = 0.000$), whereas starchy vegetable intakes fell in all arms ($P = 0.000$ for IW and control, $P = 0.003$ for *I*). Consumption of fruit increased in both intervention arms over the 3 months, although this was significant in the IW arm only ($P = 0.018$). This increase mirrored a rise in fresh/canned fruit intakes of the intervention arms ($P = 0.000$ for IW, $P = 0.002$ for *I*), which was significantly higher in the *I* arm than the control arm at 3 months ($P = 0.016$). There was a reduction in consumption of dairy/alternatives by over half of a serve in all study arms over the 3 months ($P = 0.000$). Despite this, intakes of lower fat dairy foods increased in all arms, although this was significant in the IW arm only ($P = 0.005$). Intakes of higher fat dairy foods fell in all arms ($P = 0.000$), as did medium fat dairy foods ($P = 0.000$ for IW and *I*, $P = 0.002$ for *C*).

Table 1 Median (interquartile range) number of food group serves at baseline and 3 months in each study arm: intervention + walnuts (IW), intervention (*I*) and control (*C*)

Australian Dietary Guidelines (ADG) food group ADG subgroup	Baseline				3 months			
	IW (n = 103)	*I* (n = 97)	*C* (n = 93)	*P*-value†	IW (n = 103)	*I* (n = 97)	*C* (n = 93)	*P*-value†
Grain (cereal) foods	5.2 (3.7–6.4)	5.3 (3.9–6.4)	5.3 (3.5–6.8)	0.929	3.8 (2.9–5.0)***	4.2 (3.4–5.2)***	4.0 (3.0–5.7)***	0.150
Wholegrain/higher fibre cereals/grains	1.9 (1.1–3.0)	1.6 (0.8–2.7)	1.7 (0.9–2.5)	0.461	2.3 (1.2–3.2)	2.2 (1.5–3.0)**	1.8 (0.8–2.9)	0.035‡
Refined/lower fibre cereals/grains	2.8 (2.0–4.4)	3.3 (2.1–4.6)	3.2 (2.1–4.6)	0.679	1.4 (0.7–2.5)[a],***	1.7 (0.8–2.6)[a,b],***	2.0 (1.3–3.4)[b],***	0.001
Vegetables and legumes/beans	4.4 (3.2–5.6)[a]	4.9 (3.7–6.8)[a,b]	5.2 (3.8–6.5)[b]	0.016	5.5 (4.2–6.8)***	5.3 (4.0–7.1)	5.0 (3.4–6.5)	0.400
Green and brassica vegetables	0.8 (0.5–1.3)	1.0 (0.6–1.5)	1.0 (0.6–1.5)	0.251	1.2 (0.7–1.8)***	1.1 (0.8–1.8)	1.0 (0.7–1.4)	0.146
Orange vegetables	0.4 (0.2–0.6)	0.4 (0.2–0.7)	0.4 (0.3–0.7)	0.711	0.4 (0.2–0.8)	0.4 (0.2–0.8)	0.4 (0.2–0.8)	0.831
Starchy vegetables	0.8 (0.5–1.6)	0.8 (0.4–1.6)	1.1 (0.6–1.7)	0.193	0.6 (0.2–1.0)***	0.6 (0.2–1.2)**	0.7 (0.3–1.1)***	0.526
Legumes as a vegetable	0.0 (0.0–0.3)	0.1 (0.0–0.3)	0.0 (0.0–0.3)	0.428	0.1 (0.0–0.4)	0.1 (0.0–0.5)	0.0 (0.0–0.3)	0.818
Other vegetables	1.6 (0.9–2.5)	1.7 (1.1–2.6)	1.9 (1.0–2.6)	0.247	2.2 (1.4–3.2)***	2.5 (1.5–3.1)*	1.9 (1.0–3.1)	0.203
Fruit	1.2 (0.6–2.0)	1.2 (0.7–2.1)	1.4 (0.6–2.2)	0.740	1.6 (0.9–2.1)*	1.7 (1.0–2.2)	1.4 (0.8–1.9)	0.150
Fresh/canned fruit	0.7 (0.3–1.2)	0.9 (0.4–1.5)	0.9 (0.4–1.5)	0.292	1.4 (0.8–1.8)[a,b],***	1.4 (0.9–1.9)[a],**	1.0 (0.6–1.7)[b]	0.016
Dried fruit	0.0 (0.0–0.3)	0.1 (0.0–0.3)	0.0 (0.0–0.2)	0.387	0.0 (0.0–0.1)**	0.0 (0.0–0.2)*	0.0 (0.0–0.1)	0.712
Fruit juice	0.0 (0.0–0.3)	0.0 (0.0–0.1)	0.0 (0.0–0.3)	0.554	0.0 (0.0–0.0)**	0.0 (0.0–0.0)*	0.0 (0.0–0.1)	0.047‡
Milk, yoghurt, cheese/alternatives	2.1 (1.5–3.1)	2.4 (1.7–3.2)	2.3 (1.5–3.2)	0.430	1.6 (1.0–2.2)***	1.6 (1.0–2.3)***	1.7 (1.2–2.4)***	0.413
Higher fat (>10%) dairy foods	0.6 (0.4–0.9)[a]	0.9 (0.5–1.3)[b]	0.7 (0.4–1.2)[a,b]	0.023	0.2 (0.0–0.4)[a],***	0.3 (0.1–0.6)[a],***	0.5 (0.2–0.9)***	0.000
Medium fat (4–10%) dairy foods	0.3 (0.0–0.9)	0.4 (0.0–1.2)	0.3 (0.0–1.0)	0.755	0.0 (0.0–0.4)***	0.1 (0.0–0.6)***	0.1 (0.0–0.5)*	0.317
Lower fat (<4%) dairy foods	0.6 (0.1–1.4)	0.4 (0.0–1.1)	0.5 (0.0–1.5)	0.722	1.0 (0.4–1.6)[a],**	0.7 (0.2–1.2)[a,b]	0.7 (0.0–1.2)[b]	0.018
Meats, poultry, fish, eggs, tofu, nuts, seeds and legumes/beans/tofu	2.9 (2.3–4.1)	3.1 (2.5–4.1)	3.2 (2.5–4.4)	0.377	3.1 (2.6–4.0)[a]	2.7 (2.1–3.3)[b],***	2.8 (2.3–3.8)[a,b],*	0.001
Red meat, lean (<10% fat)	0.8 (0.6–1.3)	0.8 (0.5–1.4)	0.8 (0.4–1.4)	0.995	0.5 (0.3–1.0)***	0.6 (0.3–1.0)***	0.7 (0.5–1.2)	0.091
Red meat, non–lean (≥10% fat)	0.1 (0.0–0.3)	0.2 (0.0–0.5)	0.2 (0.0–0.5)	0.248	0.0 (0.0–0.2)**	0.0 (0.0–0.2)***	0.1 (0.0–0.3)**	0.191
Poultry, lean (<10% fat)	0.6 (0.3–0.9)	0.7 (0.3–1.0)	0.6 (0.2–1.0)	0.463	0.6 (0.2–0.8)	0.6 (0.3–1.0)	0.6 (0.3–1.0)	0.284
Poultry, non–lean (≥10% fat)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.749	0.0 (0.0–0.0)*	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.111
Fish and seafood	0.2 (0.0–0.4)	0.2 (0.1–0.4)	0.2 (0.1–0.4)	0.970	0.3 (0.1–0.5)*	0.3 (0.1–0.5)*	0.2 (0.1–0.5)	0.402
Eggs	0.1 (0.0–0.2)	0.1 (0.0–0.1)	0.1 (0.0–0.2)	0.211	0.1 (0.0–0.1)[a],**	0.1 (0.0–0.1)[a,b]	0.1 (0.0–0.2)[b]	0.021
Legumes as meat alternative	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.395	0.0 (0.0–0.2)	0.0 (0.0–0.2)	0.0 (0.0–0.2)	0.807
Nuts and seeds	0.4 (0.0–0.9)	0.4 (0.1–0.7)	0.5 (0.1–0.9)	0.229	1.0 (0.9–1.2)***	0.2 (0.0–0.5)[a],**	0.3 (0.1–0.9)[a]	0.000
Unsaturated spreads and oils	3.3 (2.2–5.3)	3.8 (2.6–5.6)	3.9 (2.8–5.8)	0.074	4.6 (3.5–5.9)[a],**	2.5 (1.5–3.8)[b],***	3.0 (2.0–4.7)[c],**	0.000
Unsaturated spreads	0.1 (0.0–0.4)	0.2 (0.0–0.6)	0.1 (0.0–0.4)	0.591	0.0 (0.0–0.2)***	0.0 (0.0–0.3)***	0.0 (0.0–0.4)	0.146
Unsaturated oils	1.5 (0.9–2.5)[a]	1.8 (0.9–2.8)[a,b]	2.1 (1.2–3.0)[b]	0.041	0.9 (0.5–1.6)[a],***	1.1 (0.6–1.7)[a,b],***	1.3 (0.7–1.8)[b],***	0.044
Nuts	1.2 (0.2–2.7)	1.2 (0.3–2.2)	1.6 (0.5–2.8)	0.229	3.0 (2.8–3.7)***	0.8 (0.2–1.5)[a],**	1.0 (0.4–2.7)[a]	0.000

[a,b,c]Significant difference between groups after Bonferroni adjustment. $P < 0.05$ indicates significant differences between arms; asterisks indicate significant changes over time.

*$P < 0.05$ (Wilcoxon signed rank test).

**$P < 0.01$ (Wilcoxon signed rank test).

***$P < 0.001$ (Wilcoxon signed rank test).

†Kruskal–Wallis test.

‡Difference between groups not significant after Bonferroni adjustment.

Additionally, intakes of higher fat dairy foods were significantly lower in the intervention arms than the control arm at 3 months ($P = 0.000$), whereas lower fat dairy food intakes were significantly higher in the IW arm than the control arm at 3 months ($P = 0.000$). There was a reduction in consumption of meat/protein foods by almost half of a serve in the *I* ($P = 0.000$) and control ($P = 0.041$) arms over the 3 months. Within the meat/protein foods food group, intakes of red meat fell in all arms, whereas intakes of nuts and seeds rose in the IW arm ($P = 0.000$), which was mirrored in overall meat/protein intakes in this arm. Although sitting outside of the ADG five food groups definition, consumption of unsaturated spreads/oils was also noted to increase in the IW arm ($P = 0.003$) with a rise in nut intakes ($P = 0.000$).

The number of ADG food group serves consumed per day (see Supporting information, Figure S2) may be compared with the number of ADG food group serves per MJ total energy intake (see Supporting information, Figure S3). The reductions in consumption of grains and dairy/alternatives in all arms were attenuated when expressed relative to total energy, whereas increases in intakes of vegetables and fruit in the intervention arms and meat/protein foods in the IW arm were enhanced. Relative vegetable intakes rose in all arms ($P = 0.000$ for IW and *I*, $P = 0.018$ for control), relative fruit intakes rose in the intervention arms ($P = 0.000$), and relative meat/protein intakes rose in the IW ($P = 0.001$) and control ($P = 0.001$) arms. Relative meat/protein intakes were significantly higher in the IW arm than the other arms at 3 months ($P = 0.001$).

A decrease in the proportion of participants meeting the recommendation for grains ($P = 0.000$) and dairy/alternatives ($P = 0.001$ for IW, $P = 0.003$ for *I*, $P = 0.001$ for control) mirrored the fall in intakes of these food groups observed above (Fig. 2). The proportion of participants meeting the recommendation for vegetables increased in both intervention arms, although this was significant in the IW arm only ($P = 0.000$). The rise in the proportion of participants meeting the recommendation for fruit in the intervention arms was not significant. Finally, the proportion of participants meeting the recommendation for meat/protein foods increased slightly in the IW arm but fell in the other arms, being significant in the *I* arm only ($P = 0.003$), and the proportion was significantly lower than the IW arm at 3 months ($P = 0.009$).

Discussion

This secondary analysis of HealthTrack data demonstrated the application of the ADG database in the clinical trial context. Using this database, dietary data (often reported in composite dishes) could be categorised into the relevant and discrete food groups linked to the ADG upon which the clinical trial dietary advice was based.

Applying the ADG database provided insights into food group consumption, and, importantly, the quality of foods consumed within these groups, during the HealthTrack trial. The ADGs present dietary targets in terms of the number of food group serves per day and food quality recommendations within each of these food groups [2]. The ADG database allowed improvements to be identified at the sub-group level even when there was no apparent improvement at the broad food group level. The hierarchical classification structure of the database allows for multilevel analyses and is sensitive to changes in intake of food types (e.g. wholegrains, refined grains) within food groups (e.g. grains).

In terms of the five food groups, all participants commenced the trial with higher median vegetable intakes than average Australian adults [11] aligning with previous research suggesting overweight/obese women are already reaching this target [18]. The slight fall in vegetable intakes in the control arm may partially be explained by even higher baseline intakes than the intervention arms. This is seen in previous studies where changes in fruit and vegetable intakes are influenced by baseline intakes [19,20]. The universal fall in intakes of starchy vegetables, as a result of a reduction in consumption of potato-based discretionary foods, was consistent with our previous analysis [21]. Changes in consumption of fruit followed a similar pattern to vegetables and greater improvements were seen in the intervention arms. These changes may suggest that dietetic advice was also more effective in conveying *how* to incorporate these foods, beyond the standard AGHE specifying *which* foods to consume and in what *quantities*. These results highlight that the value of the ADG database was in providing information at multiple levels within the food groups and aids in understanding dietary changes that may not be visible at the broad food group level.

The situation for the changes in grains and dairy food consumption was not expected. A reduced proportion of participants met the recommendations for these food groups over the course of the study, in all study arms. Median intakes at 3 months showed a similar shortfall to that of the average Australian adults consumption patterns [11]. Because HealthTrack was primarily a weight loss intervention, the significant reduction in total energy intakes over 3 months [13] could have reflected intakes of these two food groups, even taking reductions in discretionary food consumption into account [21,22]. Furthermore, previous research from this team has shown that rather than switching to a higher quality dairy products

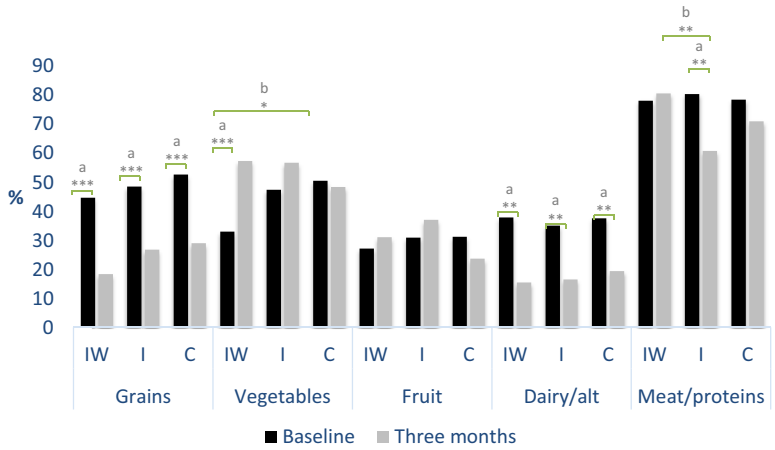

Figure 2 Proportion (%) of participants meeting the recommended number of food group serves at baseline and 3 months in each study arm: intervention + walnuts (IW), intervention (*I*) and control (*C*). [a]Significant difference between time points within arm (McNemar's test); [b]Significant difference between arms at given time point (chi-squared test). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

male participants opted to eliminate them completely [23]. Despite this, improvements in the quality of the grains and dairy/alternative foods reportedly consumed were seen through a shift from refined grains to wholegrains and higher/medium fat to lower fat dairy foods at the subgroup level. It again highlights the value of the ADG database in distinguishing food quality at the sub-group level.

The value of the database analysis was extended to highlighting changes in consumption of meat and protein-rich foods. The substantial increase in the consumption of protein in the IW arm could be attributed to the provision of 30 g of walnuts per day. Limiting red meat consumption [2] was most notable in the intervention arms, where a broad knowledge of food could have been drawn upon to discuss alternatives. Caution, however, was required when interpreting results for meat alternatives because two ADG subgroups, legumes and nuts, each contribute to more than one core food group. Legumes are classified as both a vegetable and a meat/protein, and nuts are classified as both a meat/protein and a fat with varying serving sizes. This could have resulted in an over-estimation of intakes, which did not change substantially when considering adherence to ADG at a sub-group level.

Although adherence to dietary guidelines is measured in absolute terms, ADG food group serves per MJ total energy provides a relative measure of food group intakes. Even though absolute intakes of grains and dairy fell in all arms, this effect was attenuated and in some cases reversed when expressed relative to total energy (see Supporting information, Figure S3). Relative intake increases in vegetables, fruit and meat/protein foods were more often a greater magnitude in the intervention arms, aligning with observed trends in intakes of food group serves per day.

It should be noted HealthTrack was primarily a weight loss trial, where dietary modelling for weight loss may have differed from the ADG Foundation Diet specifications. This could have an impact on the assessment of adherence to food group recommendations. The predominantly non-smoking, well-educated female sample is not representative of the wider population. The diet history method of dietary assessment can be prone to memory bias [24] which may have influenced the findings. The combination of an interviewer administered method and a primarily female, overweight sample may have also been influenced by social desirability bias in the reported food items [25]. However, given the analyses were considered in relation to energy and time point and the present study aimed to address change in food group serves of the arms of the trial this is not expected to have a substantial impact upon the outcomes. Furthermore, the ADG database application was focused on food group reporting and did not capture the changes to discretionary food items that would also have been influenced by the dietetic intervention occurring within the HealthTrack trial. Although the 3-month timeframe of the present study limits findings to the shorter-term, intervention intensity and volunteer participation were greatest during this time interval, likely amplifying the effects.

In conclusion application of the ADG database allows for food group analyses of dietary data and insight into changes at the sub-group level, enabling analysis of ADG adherence. Applying the ADG database to HealthTrack trial data demonstrated that dietitian lead individualised advice was more efficacious than usual care in promoting adherence to dietary guidelines in overweight adults.

Furthermore, benefits were observed after the provision of a healthy food supplement. Future food group analyses of dietary trial data using the ADG database could distinguish five food group intakes from discretionary sources to gain greater insights into changes in dietary patterns.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with STROBE guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned (ANZCTRN 12614000581662) have been explained.

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Conflict of interest, source of funding, authorship

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EN and YP were responsible for the study design. EN, YP and KZ were responsible for data management. LT were responsible for management of the original study. KZ was responsible for data analysis. KZ, EN and YP were responsible for the interpretation of the outcomes. EN and YP were responsible for the supervision of KZ. KZ was responsible for drafting the manuscript. EN, YP, KZ and LT were responsible for manuscript review. KZ, EN, YP and LT approved the final version of the manuscript submitted for publication.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Baseline characteristics of the HealthTrack Study participants included in the present study in each study arm: intervention + walnuts (IW), intervention (*I*) and control (*C*).

Figure S1. Flow of participants through the Health-Track Study. Participants included in the present study are indicated in grey.

Figure S2. Median number of Australian Dietary Guidelines food group serves per day at baseline and 3 months in each study arm: intervention + walnuts (IW), intervention (*I*) and control (*C*).

Figure S3. Median number of Australian Dietary Guidelines food group serves per MJ total energy intake at baseline and 3 months in each study arm: intervention + walnuts (IW), intervention (*I*) and control (*C*).

DIETARY INTAKES

The influence of sleep health on dietary intake: a systematic review and meta-analysis of intervention studies

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Abstract

Background: Poor dietary intake increases disease risk, and poor sleep influences diet. This systematic review and meta-analysis of intervention studies aimed to evaluate the effect of sleep health on dietary intake in adults.
Methods: Five online databases were used to identify studies published between 1970 and 2019. Included studies were interventions that modified sleep and reported dietary outcomes.
Results: Fifty four full texts were assessed and 24 publications were included. Following risk of bias appraisal, data were narratively summarised and a sub-group of studies (n = 15) was meta-analysed to determine the effect of sleep on dietary intake. One intervention modified sleep timing and 23 modified duration. Sleep duration was partially restricted (≤5.5 h night^{-1}) (n = 16), totally restricted (n = 4), partially and totally restricted (n = 1), and extended (n = 2). Dietary outcomes were energy intake (n = 24), carbohydrate, fat, protein intake (n = 20), single nutrient intake (n = 5), diet quality (n = 1) and food types (n = 1). Meta-analysis indicated partial sleep restriction results in higher energy intake in intervention compared with control [standardised mean difference (SMD) = 0.37; 95% confidence interval (CI) = 0.21–0.52; P < 0.001], with a mean difference of 204 kcal (95% CI = 112–295; P < 0.001) in daily energy intake, and a higher percentage of energy from fat, protein, carbohydrate (fat: SMD = 0.33; 95% CI = 0.16–0.51; P < 0.001; protein: SMD = 0.30, 95% CI = 0.12–0.47, P = 0.001; carbohydrate: SMD = 0.22, 95% CI = 0.04–0.39, P = 0.014).
Conclusions: Partial sleep restriction with duration of ≤5.5 h day^{-1} increases daily energy intake, as well as fat, protein and carbohydrate intake. Further research is needed to determine the relationship between other dimensions of sleep health and dietary intake.

Introduction

Poor dietary intake is characterised by an excessive intake of nutrient-poor foods and beverages that are high in energy, saturated fat, added sugars and sodium, and/or an inadequate intake of core foods such as vegetables, fruits, grain foods, dairy foods and protein-rich foods [1–5]. Poor dietary intake significantly increases the risk of developing obesity and chronic diseases such as type 2 diabetes and coronary heart disease [1–5]. Recently, experimental evidence has emerged to support the hypothesis that poor sleep, specifically short sleep duration, plays a causal role in poor dietary intake [6–12]. A number of studies have reported that short sleep

duration is associated with increased energy intake (EI) as a result of increased meal/snack consumption, increased energy consumption during late night hours and a tendency to choose energy-dense foods [12]. A previous systematic review and meta-analysis of randomised controlled trials (RCTs) conducted in controlled laboratory conditions reported increased EI of 253 kcalday^{-1} during partial sleep restriction (PSR) (n = 8 studies) compared to normal sleep duration [13]. The review reported mixed findings on macronutrient intake, although it did not explore free-living studies or conduct a meta-analysis on macronutrients, which are important dietary influences on health as a result of the varied roles that carbohydrate, fat and protein play in the body (e.g. provision of energy, absorption of fat-soluble vitamins, synthesis of body proteins) [14]. An additional review that did include a meta-analysis of experimental studies conducted in both laboratory and free-living settings reported that, in comparison with normal sleep duration (>6.9 h), a shorter sleep duration (≤5.5 h) resulted in increased EI of 385 kcal day^{-1} and increased fat intake (as a percentage of total energy), accompanied by lower protein intake [15]. Similarly, a systematic review of predominantly laboratory-based RCTs that modified sleep duration reported that shorter sleep resulted in increased food intake compared to normal duration [16].

These previous reviews limited their search to only studies restricting sleep duration and provided important knowledge about the effect of the quantity or duration of sleep on energy and macronutrient intake. However, sleep health is multi-dimensional and indicators of sleep health include sleep efficiency, timing, alertness during waking hours, and subjective satisfaction, as well as duration [17], some of which are potentially modifiable. Each dimension of sleep health is also associated with varying health outcomes including diet-related conditions such as coronary heart disease, diabetes, and obesity [17]. Therefore, an examination of the effect of indicators of sleep health, such as timing and quality or duration, on dietary intake in experimental studies where causal relationships can be established is important, although it is currently unclear from the literature. Furthermore, to-date the dietary outcomes examined in existing reviews and meta-analyses of sleep interventions have primarily been limited to EI and macronutrients (carbohydrate, protein and total fat). Examination of the consumption of other important nutrients, food types and overall diet quality in response to poor or improved sleep health has not been systematically synthesised. Given the association between excessive or inadequate intake of some nutrients (e.g. sodium, dietary fibre), poor diet quality, and disease risk [18,19], as well as the higher healthcare costs associated with poor diet quality [20], investigating whether sleep health influences diet quality in experimental research may provide important insights. Therefore, the aim of this review is to systematically synthesise and quantify the available evidence with respect to the effect of sleep health on a range of aspects of dietary intake, in adult populations in a range of settings (laboratory, free-living and mixed). The findings from this review may provide further evidence for a causal relationship between poor sleep health and poor dietary intake.

Materials and methods

This systematic review adheres to the PRISMA statement [21] (see Supporting information, Table S1) and the protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO: CRD42018111622). Ethics approval was not required for this systematic review.

Study criteria

To be eligible for inclusion in this review, a study had to be conducted in healthy adults (≥18 years) who were free of any medically-diagnosed health conditions, sleep disorders (e.g. sleep apnoea, insomnia), eating disorders (e.g. anorexia, bulimia nervosa), mental health conditions, diabetes, cancer and cardiovascular disease. Eligible studies were those reporting an intervention that modified one or more dimension(s) of sleep health or promoted sleep hygiene practices, and reported dietary intake, which may include (but not be limited to) EI, macronutrient intake, food types, core/non-core food intake or diet quality, at baseline, during or post-intervention. RCTs, randomised/non-randomised cross-over studies and pre-post studies conducted in both free-living, laboratory and mixed settings were included. Reviews, meta-analyses, observational studies, case studies, editorials and conference abstracts were excluded.

Search strategy

Five databases (Medline, EMBASE, Cochrane, SCOPUS and CINAHL) were searched from their inception to November 2019 using predefined keywords (see Supporting information, Table S2). Limits were applied to include human studies published in the English language. A manual search of the reference lists of relevant publications was conducted to identify any other relevant articles.

Study selection

Duplicates and articles published before 1970 were removed because, collectively, these databases report that they reliably index records from 1970 onwards (see Supporting information, Figure S1). The remaining title and abstracts were imported into Covidence [22] for screening by two independent reviewers (SF, research assistant) using the predetermined inclusion/exclusion criterion. Full texts were retrieved and evaluated against the inclusion/exclusion criteria independently. Discrepancies were resolved by a third independent reviewer (TB or MD). Consensus was reached for all included articles.

Risk of bias assessment

Included articles were assessed for risk of bias by two independent reviewers (SF, JS) using the Academy of Nutrition and Dietetics Quality Criteria Checklist for Primary Research standardised tool [23] (see Supporting information, Table S3). This 10-point checklist assesses population bias, study blinding, description of the intervention and assessment tool, statistical methods, and study funding. For each individual study, the quality of evidence was rated as being absent, present or unclear for each outcome. An overall quality rating was determined, with each study being rated as positive (four validity criteria plus ≥1 additional criteria met), negative (overall ≤4 validity criteria met) or neutral (overall four criteria met, but ≤4 validity criteria met). In cases of discrepancies regarding quality ratings a third independent reviewer was consulted (TB) and consensus was reached. No studies were excluded based on overall quality ratings.

Data extraction

Data extraction from full text was conducted using a standardised tool developed for this review, which was pilot tested on six articles and refined following evaluation. Data relating to study design, setting and country, study participants, methodology (intervention type and duration), assessment methods, outcomes assessed, and results and their significance were extracted by one reviewer (SF) and checked by a second reviewer (research assistant) (see Supporting information, Tables S4 and S5). For the purpose of this review, the study settings in which the sleep intervention took place were classified as laboratory-based, free-living or mixed. Dietary intake outcomes were classified as EI (kcal, MJ), macronutrient intake (%EI, kcal or g), single nutrient intake (mg or g), food types (kcal or g), core food group intake (%EI), non-core food intake (%EI), caffeine (mg) and diet quality (score). Mean (SD or SEM) values for daily EI and macronutrient intake for the intervention and control sleep conditions were recorded. *P*-values were recorded where possible. For studies where outcome data were not reported numerically but displayed graphically, the authors were contacted by e-mail to provide the numerical data. Where no responses were received graphs were exported to an online web digitiser to obtain numerical values (WebPlotDigitizer; https://automeris.io/WebPlotDigitizer) [24]. WebPlotDigitizer is an effective method for collecting data with high levels of intercoder reliability and validity [25]. Where SEM was reported, it was converted to SD for inclusion in the meta-analysis.

Statistical analysis

The results are summarised narratively and also quantified using a meta-analysis. In a sub-group of studies, EI was meta-analysed as average kcal day^{-1} and macronutrients as %EI. Studies reporting the mean (SD/SEM) for both intervention and control groups were included. Sufficient data were available to meta-analyse the effect of PSR on EI in 15 studies and macronutrient intake (carbohydrate, fat, protein) in 13 studies. For two studies, data were presented for sub-groups (males/females [26]; overweight/healthy weight groups [27]) and were entered into the meta-analysis separately. One study was excluded from the meta-analysis because the study participants were placed on a calorie-restricted diet during the intervention period [28]. Data were analysed using CMA, version 3 (Comprehensive Meta-Analysis, Englewood, NJ, USA). The standardised mean difference (SMD) and pooled mean difference (MD) between the intervention and control were calculated for EI (kcal day^{-1}) and macronutrient intake (%EI) and then weighted according to the sample size via the random effects model. $P < 0.05$ was considered statistically significant. In the study that reported EI in MJ, EI was converted to kcal (1 MJ = 239 kcal). In studies where macronutrient intake was reported in g or kcal, mean intake was converted to %E (mean intake/total EI). Macronutrient SD was calculated for the percentage of total EI using the delta method [29] to calculate the estimated variance (equation provided in the Supporting information, Figure S2); the variance for the ratio incorporates the mean (SD) for the macronutrient and total nutrient intake, and their covariance (estimated using Pearson correlation across studies).

Exploratory sub-group analyses were conducted by study setting (laboratory, free-living and mixed), by time point of the outcome assessment (during intervention and post-intervention) and by the number of nights of sleep restriction (1 = 1 night, 2 = 2–4 nights, 3 = ≥5 nights). The length of the intervention period (number of

nights of sleep restriction) was chosen for analysis rather than the hours (duration) of restricted sleep because all included studies had a sleep duration of <5.5 h in the intervention condition. Between studies heterogeneity was assessed using I^2 statistics and was considered to be low, moderate or high for I^2 values of 25%, 50% and 75%, respectively [30]. Publication bias was assessed by visual inspection of funnel plot (see Supporting information, Figure S3).

Results

Search results

The search strategy identified 25 412 publications for screening (see Supporting information, Figure S1) and nine additional articles through a manual search. Following abstract screening, 54 studies were identified for full text review, from which 30 studies were excluded because they did not meet the inclusion/exclusion criteria. In total, 24 studies were included in this review.

Risk of bias

The risk of bias assessment considered 23 studies as having a positive rating and one study as having a neutral rating (see Supporting information, Table S3). The study rated as neutral did not describe the method of randomisation or method of handling withdrawals and it was unclear whether participant/researcher blinding was conducted.

Description of included studies

The 24 included articles were published in the English language between 2006 and 2019 (see Supporting information, Table S4). The studies were conducted in seven countries: USA (n = 15) [6–11,26–28,31–36], Germany (n = 3) [37–39], UK (n = 2) [40,41], Japan (n = 1) [42], Canada (n = 1), France (n = 1) and Thailand (n = 1).

Description of participants

In total, 614 participants (282 male) were included in the analyses of studies and the total sample sizes ranged from 6 to 66 [31,36]. The mean (SD) age of participants ranged from 19.0 (0.9) [34] to 41.7 (10.3) years [33]. Participants were healthy, non-smoking adults, free of any medically-diagnosed health conditions and were not taking any sleep-related medications. Four studies included male participants exclusively [6,37,39,43], four studies included only female participants [27,33,38,42] and 16 studies included both male and female participants [7–11,26,28,31,32,34–36,40,41,44,45]. Body mass index (BMI) was reported in all 24 studies and mean BMI was <25 kg m^{-2} (normal BMI range) in 16 studies [6–8,11,31,32,34,35,37,39–45] and >25 kg m^{-2} (overweight/obese BMI range) in seven studies [9,10,26,28,33,36,38]. One study compared dietary outcomes in both healthy weight and overweight participants [27], one study compared dietary outcomes by sex [26] and one study compared dietary outcomes for groups with different sleep timing [44].

Description of interventions

Included studies were 11 randomised cross-over studies [6,9,11,28,33,36,37,42–45], five non-randomised cross-over studies [8,27,35,39,40], five RCTs (two-arm) [7,10,31,32,41] and three pre-post studies [26,34,38]. Of the 24 studies, 13 were conducted in a laboratory [6–11,26,28,31–33,36,37], six in a free-living setting [27,34,38,41,42,45] and five in a mixed setting [35,39,40,43,44]. The studies conducted in a mixed setting were all cross-over studies where participants spent part of the experiment in a laboratory (i.e. for assessment of sleep/dietary outcomes) and part of the experiment under free-living conditions (i.e. during the control condition or for recovery sleep). This systematic search identified 23 intervention studies that modified sleep duration and one intervention study that modified sleep timing. Of the 23 studies that modified sleep duration, four studies imposed total sleep restriction (TSR) [32,35,37,40], 16 studies imposed PSR (duration <5.5 h night^{-1}) [6–11,26–28,33,34,38,39,42–44], one study imposed both PSR and TSR [31] and two studies extended time in bed (by 1–1.5 h night^{-1}) to increase sleep duration [41,45]. In the 16 studies that partially restricted sleep duration, this was achieved by delaying bedtime and keeping wake-time consistent in seven studies [7,10,26,27,33,39,42], delaying bedtime and advancing wake-time in six studies [6,8,9,11,28,43] and by delaying bedtime and advancing wake-time on different nights in one study [44]. Two studies did not describe how bedtime/wake-time was modified to achieve restricted sleep duration. The one study that modified sleep timing delayed sleep by 3.5 h (to 03.30 AM) while maintaining a sleep duration of 8 h [36]. No studies modified sleep quality or efficiency as the intervention.

Of the 16 studies that assessed PSR compared to a control, 13 studies restricted sleep for ≤5 nights and three studies restricted sleep for >5 nights. The shortest amount of time spent in the intervention condition was one night (n = 4 studies) and the longest was 14 days (n = 2 studies). In the study that partially restricted sleep and totally restricted sleep [31], sleep was partially restricted for 5 nights and totally restricted for 1 night. In three of the four studies that assessed TSR in comparison to a control, participants spent 1 night in each condition [32,37,40]. The

other study compared one night of TSR with 2 nights in the control condition [35]. In the two sleep extension (SE) studies, the intervention periods were 2–4 weeks [41,45]. The single study that altered sleep timing compared eight nights in the intervention condition with eight nights in the control condition. A pre-intervention sleep period of one to seven nights was specified in 15 studies [7,8,10,26,28,31–34,37–39,41,43,44], of which nine were laboratory-based. The 16 cross-over studies [6,9,11,27,28,33,35–37,39,40,42–45] had washout periods ranging from 5 days [43] to 3 months [9,28]; however, two studies did not report a washout period [8,35].

Description of assessment methods

Sleep

Polysomnographic measurement was the most common method used to assess sleep (n = 13 studies) [6–11,26,28,32,33,37,39,44]. Other assessment methods included accelerometry (n = 9 studies) [10,26,27,32,35,40–42,45], self-reported sleep diaries (n = 4 studies),[27,34,41,43] self-reported sleep questionnaires (n = 3 studies) [41,42,45], heart-rate monitoring (n = 1 study) [38] and monitoring by trained laboratory staff (n = 1 study) [31]. A number of studies used a combination of methods to assess sleep (e.g. accelerometry and self-reported sleep diary).

Dietary intake

In 23 studies, *ad libitum* food intake was assessed, where participants had access to as much food as they would like [6–11,26,27,31–45]. Participants accessed food by several methods, including self-selection from laboratory menus (n = 9) [7,10,11,26,31,32,36,39,44], from outside the laboratory (i.e. food brought in from elsewhere) or under free-living conditions (n = 9) [7,11,27,34,38,41–43,45], from food provided via buffets in laboratories (n = 9) [6,33,35,37,39,40,42–44] and from food prepared by laboratory staff at scheduled meals (n = 2) [8,9] (see Supporting information, Table S5).

The length of dietary intake assessment varied across the studies. Three studies assessed one meal following each sleep condition [33,37,40] and three studies assessed 1 day of dietary intake [11,35,36]. Eight studies assessed dietary intake for 2–4 days during the sleep conditions [6,27,32,34,39,42–44], whereas 10 studies assessed dietary intake for ≥5 days [7–10,26,28,31,38,41,45]. Weighed food record was the most common dietary assessment method used, performed by laboratory assistants (n = 13 studies) [6,9–11,26,28,31–33,36,39,40,44] and by study participants self-reporting food intake (n = 2 studies) [27,43]. Self-reported food diaries of 2–9 days (n = 5 studies) [34,38,41,42,45] and food records obtained from hospital logs and food inspections (n = 2 studies) [7,35] were also used to assess dietary intake. The dietary assessment method was unclear in two studies [8,37]. Dietary intake was most commonly calculated from the food records and diaries using nutrition analysis software (n = 18 studies) [6,8–11,26–28,31,32,34,36,38,39,41,43–45], whereas two studies calculated intake from food labels [35,42], one reported the total weight of foods [40] and three studies did not report the method by which intake was calculated [7,33,37].

Description of outcomes

The results of the interventions are summarised in the Supporting information (Table S6).

Sleep outcomes

In the studies that partially restricted sleep duration, the mean (SD) sleep duration in the intervention condition ranged from 3.77 (0.64) h [11] to 5.49 (0.59) h [38]; however, three of these studies did not report the sleep duration [10,26,31]. The mean (SD) sleep duration in the control condition of the PSR studies ranged from 6.4 (1.7) h [34] to 9.02 (0.96) h [38]. Three of the four studies that imposed TSR reported the mean (SD) duration of sleep in the control condition, which ranged from 6.97 (0.13) h [37] to 7.6 (1.1) h [35]. In the two SE studies, one study reported an increase of 36 min night^{-1} during the intervention condition [45], whereas the other reported a mean difference in sleep duration of 32 min night^{-1} between the intervention and control groups [41]. The two SE studies also reported change in Pittsburgh Sleep Quality Index global scores [46]. One reported a mean difference between the control and intervention condition of −0.47 [95% confidence interval (CI) −1.63 to 0.69], favouring the intervention [45], and the other study reported a mean difference in the change from baseline of −1.3 points (95% CI = −2.3 to −0.3) (Change from baseline, control 0.3, intervention −1.1 points) [41]. One SE study reported a mean difference in the change from baseline of Sleep hygiene index score of −4.9 (control 1.0, intervention −3.9) [41]. The mean difference in Epworth sleepiness scale score between the control and intervention condition in one SE study was reported to be −0.99, favouring the intervention condition [45]. The single study that altered sleep timing reported prescribed sleep times of 3.30 AM to 11.30 AM (intervention) and midnight to 8.00 AM (control) [36].

Dietary outcomes

Of the included studies, EI was reported in all 24 studies, expressed as kcal or MJ. Macronutrient intake (carbohydrate, total fat and protein) was reported in 20 studies [6,8–11,26,27,31–33,35–44], expressed as kcal or g of each macronutrient [6,8,11,27,35,36,40,41,44] or %EI [9,26,31–

[33,38,39,41,43]. Five studies reported sugar intake [11,31,35,36,41], four studies reported saturated fat intake [11,31,36,41] four studies reported fibre intake [11,31,36,41], two studies reported sodium intake [11,36], one study reported alcohol intake [41], one study reported caffeine intake [41], one study reported monounsaturated fat intake [36], one study reported polyunsaturated fat intake [36], one study reported intake of different types of foods [26] and one study reported diet quality [41].

Effect of sleep on energy intake

Sleep duration

Partial sleep restriction compared with control. In total, 16 studies (358 participants) assessed the effect of PSR compared to control on EI [6–11,26–28,33,34,38,39,42–44]. Sleep duration was restricted for one night ($n = 4$ studies), two nights ($n = 2$ studies), three nights ($n = 1$ study), four nights ($n = 2$ studies), five nights ($n = 4$ studies), eight nights ($n = 1$ study) and 14 nights ($n = 2$ studies). Nine of the studies were carried out in a laboratory setting, four under free-living conditions and three in a mixed setting. In these 16 studies, EI assessment ranged from one meal, through to 14 days of assessment in each sleep condition. Of these, nine reported a significant increase in EI [6,8–11,26,28,38,43] and seven studies reported no significant difference [27,28,33,34,39,42]. One of the studies that found no significant difference in EI placed participants on a calorie-restricted diet and expected no difference in EI between sleep conditions [28]. Another of the studies that found no significant difference in EI reported on the effect of 50% sleep duration restriction with an advanced wake-time and 50% sleep restriction with delayed bedtime, compared to control, on EI [44]. No significant difference in EI was found between restricted sleep duration with different timing and the control condition. None of the 16 studies reported a decrease in EI in response to PSR, compared to control.

The meta-analysis ($n = 15$ studies) (see Supporting information, Figure S4) indicated that participants undergoing a period of PSR consumed more energy compared to the control condition (SMD = 0.37; 95% CI = 0.21–0.52; $P < 0.001$), with a pooled mean difference of 204 kcal (95% CI = 112–295; $P < 0.001$; $I^2 = 0\%$) in total daily EI. The sub-group analysis by setting indicated a significant difference in daily EI between the intervention settings compared to the control (SMD = 0.37; 95% CI = 0.21–0.53; $P < 0.001$; $I^2 = 0\%$; MD 214 kcal; 95% CI = 119–310; $P < 0.001$), with laboratory-based interventions showing the largest difference on increased EI compared to the control (Laboratory: SMD = 0.46; 95% CI = 0.23–0.69; $P < 0.001$); Free-living: SMD = 0.27; 95% CI = 0.01–0.52; $P = 0.040$; Mixed: SMD = 0.33; 95% CI = −0.09–0.76; $P = 0.124$). A significant difference was also found when the studies were analysed by the period of sleep restriction (number of nights) compared to the control (SMD = 0.37; 95% CI = 0.21–0.52; $P < 0.001$; $I^2 = 0\%$; MD 224 kcal; 95% CI = 125–322; $P < 0.001$). PSR of five nights or more demonstrated the largest effect on increased EI (≥5 nights: SMD = 0.50; 95% CI = 0.24–0.76; $P < 0.001$; 2–4 nights: SMD = 0.34; 95% CI = 0.01–0.67; $P = 0.042$; 1 night: SMD = 0.26; 95% CI = 0.004–0.51; $P = 0.047$). A sub-group analysis found a significant difference in the time point of EI assessment (during or post-intervention) compared to the control (SMD = 0.37; 95% CI = 0.20–0.53; $P < 0.001$; MD 225 kcal; 95% CI = 114–336; $P < 0.001$). The largest difference was found when EI was assessed during the intervention (During: SMD = 0.42; 95% CI = 0.22–0.63; $P < 0.001$; Post: SMD = 0.27; 95% CI = 0.003–0.54; $P = 0.053$).

Partial sleep restriction compared with total sleep restriction. One two-arm RCT (66 participants) assessed EI for 1 day following one night of TSR and one night of restricted sleep (4 h in bed) under laboratory conditions. EI was not significantly different between the sleep conditions [31].

Total sleep restriction compared with control. Four studies (121 participants) assessed the effect of one night of TSR compared to one or two nights of the control duration (ranging from 7.0 to 7.9 h) on EI [32,35,37,40]. Two of the studies were conducted in a laboratory [32,37] and two were conducted in a mixed setting [35,40]. Following each sleep condition, one study assessed EI at a breakfast buffet [37,40] and one at an afternoon tea buffet [37]. One study assessed EI over an 18-h period (three consecutive 6 h intervals) [35] and one study assessed EI for a 3-day period [32]. None of the four TSR studies reported a significant difference in EI between the TSR and control conditions.

Sleep extension compared with control. Two studies (63 participants) assessed the effect of SE on EI [41,45]. The 4-week RCT assessed EI for 7 days and reported no significant difference between the intervention group who increased sleep duration by 21 min night^{-1} (95% CI = 0:06–0:36), and the control group [41]. The 2 week cross-over study assessed EI for 6 days and also reported no significant difference in EI when the participants increased their mean (SD) sleep duration by 36 (45.2) min night^{-1} [45].

Sleep timing

One cross-over study (five participants) conducted in a laboratory assessed EI for 1 day following four study

phases: 4 days of normal sleep and normal meal timing, 4 days of normal sleep and late meal timing, 4 days of late sleep and normal meal timing, and 4 days of late sleep and late meal timing, all with identical sleep duration. Late sleep and late meal timing were associated with reduced EI relative to normal sleep and normal meal timing [36].

Effect of sleep on macronutrient intake

Sleep duration

Partial sleep restriction compared with control. Thirteen studies (281 participants) assessed the effect of PSR compared to control on macronutrient intake (total fat, carbohydrate and protein). Four studies found a significant increase in total fat intake (measured as g and %EI), one in response to one night of PSR [43] and three studies in response to five nights of PSR [10,11,26]. Three of these studies were conducted in a laboratory [10,11,26] and one in a mixed setting [43]. The period of dietary assessment ranged from 1 to 7 days.

No significant difference in total fat intake was reported in nine studies in response to PSR [6,8,9,27,33,38,39,42]. Four of the 13 studies, with total dietary assessment ranging from 2 to 28 days, reported a significant increase in carbohydrate intake, measured as kcal, g and %EI [6,8,9,44]. One of these studies assessed the effect of 50% sleep restriction with an advanced wake-time and 50% sleep restriction with delayed bedtime, compared to a control, and reported increased carbohydrate intake (kcal) in the group that restricted sleep with delayed bedtime. Three of these studies were conducted in a laboratory, whereas the other study was under mixed laboratory/free-living conditions. Nine studies with dietary assessment ranging from one meal in each condition to 9 days found no significant difference in carbohydrate intake [10,11,26,27,33,38,39,42,43]. Protein intake following PSR compared to control was found to increase in three studies [8,10,33] and decrease in one study, measured as g and %EI [26]. All studies were conducted in laboratories and assessed dietary intake for periods of one meal in each condition through to 10 days. One study conducted in a free-living setting, comprising a normal-weight group and an obese group, found that, following PSR, protein intake (g) decreased in the normal-weight group and increased in the obese group [27]. Seven studies found PSR had no significant effect on protein intake compared with control [6,9,11,38,39,42,43].

The meta-analysis ($n = 13$ studies) (see Supporting information, Figures S5 to S7) indicated participants undergoing a PSR intervention consumed a higher percentage of energy from the three macronutrients fat, protein and carbohydrate compared with the control condition (Fat: SMD = 0.33; 95% CI = 0.16–0.51; $P < 0.001$; MD = 1.6 %EI; 95% CI = 0.7–2.5; $P < 0.001$; Protein: SMD = 0.30, 95% CI = 0.12–0.47, $P = 0.001$; MD = 0.4 %EI 95% CI = 0.1–0.8; $P = 0.006$; Carbohydrate: SMD = 0.22; 95% CI = 0.04–0.39; $P = 0.014$; MD = 0.9 %EI; 95% CI = 0.01–1.9; $P = 0.048$).

Partial sleep restriction compared with total sleep restriction. One two-arm RCT (66 participants) assessed macronutrient intake following one night of TSR and one night of restricted sleep [31]. The study reported significantly increased protein and saturated fat intake (%EI) during restricted sleep compared with TSR. Total fat and carbohydrate intake was not significantly different between the two sleep conditions [31].

Total sleep restriction compared with control. Four studies ($n = 121$ participants) assessed the effect of one night of TSR compared to one or two nights of the control duration (ranging from 7.0 to 7.9 h) on macronutrient intake. Of the four studies, two reported a significant increase in total fat intake (g and %EI) in response to TSR [32,40], whereas the other studies reported no significant difference [37]. The two studies that reported increased fat intake were conducted in a laboratory [32] and a mixed setting [40]. These studies assessed macronutrient intake for one meal following each sleep condition [40] and for 3 days [32]. One laboratory-based study reported a significant decrease in carbohydrate intake (%EI) as a result of TSR, after 3 days of dietary assessment [32], whereas the other three studies reported no significant difference in carbohydrate intake between the two sleep conditions [37,40]. No difference in protein intake was reported in the studies that assessed the effect of one night of TSR compared with control.

Sleep extension compared with control. One two-arm RCT ($n = 42$ participants) assessed the effect of 4 weeks of SE on macronutrient intake. The study reported that the change from baseline for percentage of energy from protein was significantly lower in the control group compared with the intervention group. There was no significant difference in total fat or carbohydrate intake [41].

Sleep timing

One cross-over study (five participants) conducted in a laboratory assessed EI for 1 day following four study phases: 4 days of normal sleep and normal meal timing, 4 days of normal sleep and late meal timing, 4 days of late sleep and normal meal timing, and 4 days of late

sleep and late meal timing, all with similar sleep duration. The study reported significant effects of sleep timing on total fat intake (grams), but not on protein or carbohydrate intake [36].

Effect of sleep on other aspects of dietary intake

Six of the 24 studies reported aspects of dietary intake in addition to energy, carbohydrate, total fat and protein intake [11,26,31,35,36,41]. Sugar intake, assessed in five studies [11,31,35,36,41] (184 participants) was found to be not significantly different when comparing PSR with control [11], TSR with PSR [31] and TSR with control [35], as well as when sleep timing was delayed [36] and during a period of extended sleep duration [41]. Intake of free sugars, however, which are defined as all monosaccharides and disaccharides added to foods, plus sugars naturally present in honey, syrups and unsweetened fruit juices, was assessed in one study (42 participants) and found to be significantly reduced during a period of extended sleep duration compared with control [41]. Saturated fat intake, assessed in four studies [11,31,36,41] (139 participants) was reported to be significantly higher in one study during a period of PSR compared to control [11], higher as percentage of energy in one study during the day following SR compared to TSD [31], and significantly affected by delayed sleep timing [36]. Four studies assessed fibre intake [11,31,36,41] (139 participants) and reported fibre intake to be not significantly different when comparing PSR with control [11] TSR with PSR [31], when sleep timing was delayed [36] and during a period of extended sleep duration [41]. Sodium intake assessed in two studies [11,36] (31 participants) was also reported to be not significantly different between PSR and control conditions [11] and when sleep timing was delayed [36]. One study (five participants) assessed the effect of altered sleep timing on monounsaturated and polyunsaturated fat intake and reported significant effects of sleep timing [36]. Alcohol and caffeine intake and diet quality were assessed by one study (42 participants) and were found to be not significantly different during a period of extended sleep duration compared with control [41]. One study (44 participants) assessed the effect of PSR compared with control on intake of a number of food and drink types [26]. This study reported significantly increased EI from bread, cereal, plain rice and pasta; condiments; desserts; salty snacks; and caffeine-free soda and juice during PSR compared with control, as well as no significant difference between the sleep conditions in EI from meat, eggs and fish; fruit, vegetables and salad; or milk [26].

Discussion

The present review aimed to examine the available evidence with respect to the effect of sleep health on a range of aspects of dietary intake in healthy adults. This research identified that experimental studies have predominantly examined the effect of restricted sleep duration on dietary intake, and a small number of studies have examined the effect of extended sleep duration and altered sleep timing on dietary intake. The two included SE studies also provided sleep hygiene recommendations as part of the intervention to increase sleep duration. Better sleep hygiene practices are associated with better sleep quality [47] and sleep hygiene is a component of sleep interventions [48]; however, it is not possible to disentangle the effects of the extended sleep duration and sleep hygiene on dietary intake in these studies. As such, it remains unclear how modifying sleep quality influences dietary intake. Furthermore, from the available literature, dietary intake has been predominantly measured in terms of EI and macronutrient intake, with limited reporting of other aspects of diet, such as overall diet quality. The findings suggest that sleep duration restricted to $\leq$5.5 h day^{-1} increases EI, with a mean increase of 204 kcal day^{-1}. The increased EI resulting from restricted sleep duration was found to be increased by higher fat, protein *and/or* carbohydrate intake. These findings provide up-to-date evidence that partial sleep restriction can negatively influence aspects of dietary intake, which may have implications for weight management and chronic disease risk, if not offset by increased energy expenditure. This review extends on previous research [13,15] with findings from the meta-analysis suggesting that PSR of $\geq$5 days has a greater effect on increasing EI than sleep restricted for a shorter period of time. This may be important to understand given that a 5-day working week is common in society, and individuals report a shorter sleep duration on nights preceding work-days [49,50].

The magnitude of the increased EI in response to partial sleep restriction reported in this review (204 kcal day^{-1}) is comparable with two recent systematic reviews and meta-analyses [13,15]. Zhu *et al.* [13] reported increased EI (253 kcal day^{-1}) in response to PSR compared to normal sleep (n = 8 studies); however, the current meta-analysis involves seven other studies, including those conducted under free-living conditions, as well as studies conducted under controlled laboratory conditions. Al Khatib *et al.* [15] reported increased EI of 385 kcal day^{-1} in response to PSR (n = 11 studies), as well as increased fat intake and decreased protein intake. The current meta-analysis indicated that increased protein, carbohydrate or fat intake may be responsible for the increase in EI; however, the current meta-analysis included six additional studies examining macronutrient intake.

Several mechanisms have been hypothesised as potential pathways by which inadequate sleep may increase EI

[12,51,52]. Short sleep duration can disrupt circadian rhythms and circadian misalignment has been found to disrupt appetitive hormones [53] leptin (which promotes satiety) [54] and ghrelin (which stimulates hunger) [55]. Both epidemiological and experimental studies have found that a shorter sleep duration is associated with lower leptin levels [56–58] and higher ghrelin levels [58,59]. However, a meta-analysis by Zhu *et al.* [13] did not find strong evidence to support sleep restriction influencing leptin (13 studies) and ghrelin (11 studies) levels. Currently, the interaction between sleep restriction, circadian misalignment, hormonal changes and the subsequent influence on diet is unclear. A potential neuronal mechanism has been proposed for the increased EI resulting from poor sleep. Several studies have demonstrated activation of brain regions associated with reward and food-related behaviours after a period of restricted sleep [37,60,61]. The brain regions involved in greater activity patterns have been associated with motivation, decision-making, cognitive processing and self-control, and consequently the altered neuronal activity predisposes individuals to seek food as a reward [61]. The availability of more time to eat as a result of short sleep duration and extended wakefulness is also proposed as an explanation for increased EI [62,63]. Several experimental studies have found that a shorter sleep duration results in more frequent meals and snacks [9,10,64], often with the consumption of energy-dense foods during late-night hours [10]. Consuming food, rather than sleeping, during times when the circadian system is promoting sleep and the body is experiencing reduced glucose tolerance and reduced gastric emptying rates [65] is associated with metabolic dysregulation (e.g. reduced insulin sensitivity [66] and increased blood pressure) [67]. Prolonged exposure to such metabolic dysregulation may increase the risk of obesity, type 2 diabetes and cardiovascular disease [67–69]. There is a need to identify biomarkers that identify individuals most vulnerable to overeating [70,71], and biomarkers that identify individuals at increased risk of adverse metabolic effects due to sleep restriction and circadian disruption [67,72]. Research has found large inter-individual differences in EI in response to sleep restriction [70,73] as identified in some studies included in this review, which suggests differential vulnerability to overeating during sleep restriction.

Given that 21 out of 24 included studies restricted sleep duration and also that 15–30% of adults [74] report sleep durations less than the recommended 7–9 h night^{-1} [75], the two studies identified by this review that sought to extend sleep duration provide new insights regarding how sleep duration influences dietary intake [41,45]. These studies were conducted interventions in free-living settings with a focus on sleep hygiene practices and achieved an increase in sleep duration of 21–36 min day^{-1} for periods of 2–4 weeks. This may suggest that increasing sleep duration is feasible in healthy, short-sleeping adults by addressing sleep hygiene practices. This is supported by evidence from combined physical activity and sleep health interventions that have reported improved sleep quality and also duration among physically inactive adults with poor sleep quality [76]. However, both of the included sleep duration extension studies reported no significant difference in dietary intake as a result of the extended sleep, with the exception of significantly higher percentage of energy from protein and significantly lower intake of free sugars in one study [41]. It could be argued that perhaps the increase in sleep duration was not sufficient enough to illicit change in other aspects of dietary intake, particularly because neither group achieved the recommended sleep duration of 7–9 h night^{-1} [75]. Similarly, it may be that the period of time during which participants actually slept longer for was not sufficiently long to influence change in dietary intake. Thus, further experimental studies are needed to determine how extending sleep duration among shorter sleepers may benefit dietary intake, including an examination of the time periods that potential dose–response relationships may occur over.

Strengths and limitations

The strengths of this systematic review are that it was conducted and reported according to PRISMA guidelines [21] and a comprehensive search strategy was implemented across multiple databases. Study selection, data extraction and risk of bias were completed by two independent reviewers, as is standard practice for high-quality systematic reviews. There were also a number of limitations. The review was limited to studies that were published in the English language and was also limited to a search of several online databases only; however, these were considered to comprise, as well as index, the majority of journals that would be likely to publish health-related research. With regard to the included studies, the sample sizes of the identified studies were small, ranging from six to 66, and the populations were relatively young (19–42 years). A large proportion of the interventions were conducted in a controlled laboratory setting [6–11,26,28,31–33,36,37] which allows high levels of control in terms of compliance and measurement; however, these conditions may attenuate some of the effects of sleep modification on dietary intake (e.g. the modification of sleep for short periods of time, the availability of food at specific times only). However, we acknowledge the logistical and ethical considerations associated with restricting sleep for longer periods and in less controlled settings outside the laboratory. We also acknowledge the

limitations of free-living studies, particularly the potential for non-compliance with regard to sleep instructions, and inaccurate dietary record-keeping. The results should also be interpreted with the acknowledgement that the requirement to record dietary intake may have caused participants to alter their dietary behaviour. Likewise, although the participants in most studies had access to as much food as they would like, the provision of prepared meals in laboratories that were not selected by participants or via set menus may not be reflective of real-life eating conditions and may have influenced dietary intake results. In addition, the period of dietary assessment was short in many of the studies (e.g. one meal, 1 or 2 days), capturing only a snapshot of dietary intake. The restriction of caffeine and/or alcohol in some of the interventions should also be noted, as both caffeine and alcohol can influence aspects of sleep health [77–80], and alcohol consumption can impact overall EI [81].

Future research priorities

This research identified that experimental studies have predominantly examined the effect of restricted sleep duration on dietary intake. Although it is evident that restricted sleep duration influences some aspects of dietary intake (e.g. EI), it is largely unknown how other dimensions of sleep health, which are potentially modifiable, such as sleep timing or quality, influence dietary intake. The mechanistic pathways linking changes to sleep duration and circadian rhythm to altered dietary intake are also unclear. Although some research has recently been conducted [41,45], it is also unclear how increasing sleep duration may affect dietary intake, particularly EI and overall diet quality. Furthermore, because the majority of sleep interventions to-date have been conducted over relatively short time periods, longer intervention and assessment periods, where logistically and ethically possible, may better reflect the true effect of sleep health on dietary intake. This is particularly relevant for studies that examine how improved sleep health affects dietary intake.

Through our comprehensive examination of the literature, it was also identified that the 24 included studies all reported EI, and 20 reported carbohydrate, protein and total fat intake, whereas few studies reported other important aspects of dietary intake. Reporting dietary outcomes such as energy and macronutrients is important; however, this review highlights knowledge gaps such as how sleep influences overall diet quality, as well as the consumption of nutrients and foods that are protective against chronic diseases or that may increase the risk of developing chronic diseases (e.g. dietary fibre, polyunsaturated fat, sodium) [18,19]. It is suggested that future sleep interventions undertake comprehensive dietary measurement and, to achieve this, study participants may need to be allowed broader access to food and beverages. In addition, given that many of the studies identified in this review were conducted exclusively in laboratory-based settings, extending research to include more studies conducted under free-living conditions, or mixed free-living/laboratory settings, where individuals are required to purchase, prepare and consume food as part of normal day-to-day activities, is also suggested.

Conclusions

This systematic review and meta-analysis provides further evidence that a shorter sleep duration increases EI in adults, which can be a result of total fat, protein and/or carbohydrate intake, and that, as the time period of PSR increases, EI also increases. The included studies predominantly examined restricted sleep duration, with little evidence of the effect of other dimensions of sleep health on dietary intake. The included studies also predominantly examined EI and macronutrient intake, and such research is warranted to investigate how sleep influences overall diet quality and intake of important nutrients. This review highlights the need for future experimental studies that examine the relationship between the many aspects of sleep health, including sleep quality, sleep timing and sleep duration extension, and dietary intake in adults in real-life settings where usual and comprehensive dietary intake can be assessed.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with PRISMA guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned (PROSPERO: CRD42018111622) have been explained.

Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.
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All authors developed the search and contributed to the data screening and extraction and drafting of the manuscript. All authors have critically reviewed the manuscript and have approved the final version submitted for publication.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. PRISMA-P 2015 checklist for studies included in the systematic review.

Table S2. Search strategy.

Table S3. Study quality and risk of bias assessment checklist.

Table S4. Summary table of characteristics reported in the included studies.

Table S5. Overview of the periods of sleep modification and dietary assessment, as well as dietary assessment methods, in the included studies.

Table S6. Results of the included studies assessing the impact of sleep interventions on dietary intake in adults.

Figure S1. Flow diagram of study selection.

Figure S2. Variance estimate equation.

Figure S3. Funnel plot for a meta-analysis of the effect of sleep duration on energy intake in adults.

Figure S4. Meta-analysis of the effect of partial sleep restriction on energy intake in adults.

Figure S5. Meta-analysis of the effect of partial sleep restriction on total fat intake in adults.

Figure S6. Meta-analysis of the effect of partial sleep restriction on protein intake in adults.

Figure S7. Meta-analysis of the effect of partial sleep restriction on carbohydrate intake in adults.

DIETARY INTAKES

Trends in food sources of added sugar in Australian eating patterns between 1995 and 2012 using national consumption survey data

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Abstract

Background: When aiming to develop dietary messaging to achieve reductions in added sugar intakes, it is necessary to identify key food contributors. Food contributors are not expected to remain static over time. Therefore, the present study aimed to compare the total added sugars (AS) intake and related food sources for adult respondents of two Australian national consumption surveys.
Methods: Repeated 24-h recall data from the 1995 National Nutrition Survey (1995NNS, n = 10 851) and the 2011–12 National Nutrition and Physical Activity Survey (2012NNPAS, n = 9341) was used to estimate AS consumption. Food group sources of AS were examined per consumer and per capita and the food group predictors of energy provided by AS were determined.
Results: A significant difference in total AS intake was identified by age and gender between the surveys (all $P < 0.001$). Increased variability in food group contributions per consumer was also identified. Nine of the top 20 food groups from the 1995NNS differed ($P < 0.001$) in their contribution to AS in 2012NNPAS per consumer. Fewer changes were apparent at the population level, with >40% AS coming from only three food groups. Age-stratified analyses showed that the 'sugar, honeys and syrups' and the 'sweetened beverages' food groups were the top contributors between the surveys up to the age group of 70 years. 'Sugar, honey and syrups', 'chocolate and chocolate-based confectionery,' and 'other confectionery' (all, $P < 0.001$) were significant predictors of AS intake (1995NNS, $r^2 = 0.755$; 2012NNPAS $r^2 = 0.740$).
Conclusions: At a population level, food group contributions to AS intakes for Australian adults have not changed substantially over time, yet notable shifts in AS can be seen when targeting only the consumers of these food sources. 'Cake type desserts' appear to be increasingly consumed though 'sweetened beverages' remain a major contributor to AS intakes warranting targeted public health strategies.

Introduction

The consumption of excess added sugars has been related to an increase in chronic disease risk and mortality [(1–3)]. Consumption of >25% of energy from added sugars compared to the consumption of less than 10% of energy from added sugars was associated with a substantially increased risk of cardiovascular disease mortality in a US cohort study [(2)]. However, it has been argued that the dietary sources of sugar should not be considered risk

factors unless they are consumed in excess and in combination with an increase in energy intake and/or reduced expenditure; excess weight leads to an increased risk of chronic disease [4]. An increase in dietary sugars has been associated with an increase in body weight [5,6]. A World Health Organization (WHO) report that included multiple systematic reviews and evidence profiles suggested that free sugars are associated with incidence of dental caries, as well as the displacement of nutrient rich foods, resulting in unhealthy dietary intakes that lead to the development of overweight and obesity, a risk factor for many non-communicable diseases [5]. An earlier meta-analysis of observational and intervention studies found that, in intervention trials where participants were consuming *ad libitum* intakes, there was an association between reduced total sugars and a reduction in body weight (−0.80 kg), whereas an increased consumption was related to increased body weight (0.70 kg). This same analysis also found that intervention trials including an energy equivalent exchange of carbohydrates showed no change in body weight (−0.04 kg). Analysis of observational studies included in the review resulted in an increased odds of being overweight (odds ratio = 1.55) for consumers in the highest compared to the lowest intake categories of sugar sweetened beverages [6].

In another meta-analysis of clinical trials investigating the effect of free sugars intake on cardio-metabolic risk factors, blood pressure and blood lipid profiles, an effect of free sugars was found independent of body weight. Higher, compared to lower, intakes of free sugars significantly increased total cholesterol, low-density lipoprotein, high-density lipoprotein and triglycerides, whereas the greatest effect on blood pressure was found in intervention trials that were longer than 8 weeks in duration [7]. Subsequently, a meta-analysis of the exchange of free sugars for complex carbohydrates found similar outcomes for increased blood lipids though no increase in blood pressure and no effect on body weight,[8], although there was substantial heterogeneity between the studies.

Despite some inconsistencies between studies regarding the impact of added sugars on disease risk factors, substantial interest in this area has warranted population level food intake analyses to identify dietary sources of added sugars to inform nutrition policy change [9,10]. Current evidence provided by the most recent global burden of disease study suggests that dietary messaging that targets a single nutrient rather than the food sources of that nutrient has limited success [11]. Further to this, added and free sugars cannot be chemically analysed from a food item limiting their appearance on most food labels. In turn, this challenges the ability to develop targeted public health messages for the consumer.

In the case of added sugars, there are inconsistencies in the quantification methods that have led to uncertain estimates [10,12,13]. Globally, most countries report *total* sugars intake, whereas *added* sugars intake is less frequently reported [14]. Estimates of intake for added sugars have been reported by indirect methods [10], including the use of apparent consumption data and national intake surveys [15]. Australian apparent consumption data have indicated that added sugars intake has declined over time, following a trend echoed in other high-income countries [16,17]. Although apparent consumption data provide per capita availability, they do not account for food waste, nor provide individual or sub-population intakes.

Previous studies that explored national added sugars intake have been limited to a focus on the population as a whole rather than subsets by respondent characteristics [15,17,18]. This is particularly important given that added sugars are often components of discretionary (occasional) food items of which Australians are reported to consume over 30% of total energy intake [19]. To reduce such intake patterns targeted, rather than general, essential advice is required that cannot be achieved using population level estimates. The interchangeable use of free rather than added sugars has also become apparent in nutrition messaging. Although the WHO guidelines refer to *free* sugars, which include added sugars as well as sugars in the intact forms of fruits and vegetable juices, many dietary guideline messages address *added* sugars [20]. Furthermore, dietary guidelines are the messages used within countries to guide consumer intakes, whereas WHO guidelines are used by policy makers as a point of translation for shifting intakes [21].

Nationally representative dietary intake surveys in Australia are sporadic. The most recent survey, the 2011–12 National Nutrition and Physical Activity Survey (2012NNPAS), was a component of the 2011–13 Australian Health Survey (AHS) [22]. Sixteen years prior, the 1995 National Nutrition Survey (1995NNS) was undertaken [23]. Although earlier studies provided useful cross-sectional comparisons for the intakes of Australians, heterogeneity in the methods used to estimate added sugars in the food supply has not allowed for direct comparisons [21]. Because the Australian food supply and food consumption patterns of consumers have changed substantially over time [17], the present study aimed to compare the quantity and sources of added sugars reported by adult respondents of the 1995NNS and 2012NNPAS to determine changes over time. A secondary aim was to determine the food predictors of total added sugar intake in each of the surveys. This analysis will add to the evidence with respect to identifying the food sources of

added sugars that may become drivers for future nutrition policy change.

Materials and methods

This study compared these data on intake of added sugars for adult respondents (>18 years) from two nationally representative Australian surveys: the 1995NNS (n = 10 851) [24,25] and 2012NNPAS (n = 9341) [24,26]. Both surveys applied a 24-h recall methodology that was repeated on a second occasion, where possible. The 1995NNS respondents were a subsample of volunteers recruited from the 1995 National Health Survey, including large sub-populations of individuals from very remote areas of Australia, and comprised the separate sampling of Indigenous people in the main survey. Nutritionists trained as interviewers administered the 24-h recall for the 1995NNS using a three-phased paper-based questionnaire with no visual resources for the respondents to determine the portion sizes of the reported foods. An additional food frequency questionnaire captured additional food and beverage information. The 2012NNPAS respondents were a subsample of the Australian Health Survey excluding persons from remote areas of Australia [24]. A separate health survey with a nutrition and physical activity sub-component was developed for Aboriginal and Torres Strait Islander respondents. The 24-h recall was administered by trained interviewers using a five-phased computer-based interview that used a multiple pass approach [27]. The present study uses the first day of 24-h recall data from each of the surveys. The term 'added sugars' in our analysis refers to sugars that are added to foods and beverages during manufacturing, processing or at the table, including syrups and honey [17]. It does not include fruit or fruit juices that are included under the 'free sugars' definition of WHO [15].

Re-coding and classification of food groups in 1995NNS and 2012NNPAS

A 10-step method was used to estimate added sugars intake for the two surveys. The method was applied to the AUSNUT 2011–13 food composition database that was used to analyse the 2012NNPAS [17] and included six objective followed by four subjective steps. The method has been successfully applied to longitudinal data to estimate Australian food sources of added sugars [28] and was subsequently used with the AUSNUT 1999 food composition database for the 1995NNS [29]. The two food composition databases were not directly comparable in their original form as a result of changes in food composition practices over time [30]. Differences had occurred in the coding of food items as a result of changes in data collection methods, food consumption patterns, manufacturing formulations and supply, as well as differences in data processing requirements such as food group coding and categorisation [31]. Food items in the AUSNUT databases are identified using unique eight-digit codes using nested hierarchical food group coding; the first two digits indicate the major food group, followed by three digits for the sub-major food group and the remaining five digits for the minor food group. In the present study, the three digit sub-major food group codes were used to match the food items, food groups and the coding systems between the food composition databases [32,33]. Differences in the food coding systems between the 1995NNS and 2012NNPAS required re-coding of the food items using a predetermined concordance file [32–35]. The names of the food groups are abbreviated for clearer presentation (see Supporting information, Table S1). The above methods enabled a comparison of the total intake of added sugars and the food group contributions between the databases.

Data cleaning and management

The 24-h recall data from day 1 were used because the response rates for day 2 differed between the two surveys (10% 1995NNS versus 60% 2012NNPAS) [24]. One day of data was considered sufficient following a comparison of population means [36]. The Goldberg cut-off method [37] was used to account for misreporting of intakes within the two surveys (i.e. extreme under- and over-reporting). For this, an energy intake (EI) to BMR ratio (EI:BMR) [24] was calculated using a standardised physical activity level (PAL) of 1.55 in accordance with the Australian Bureau of Statistics guidelines [24]. Respondents with a EI:BMR outside of the 95% confidence interval for PAL (0.87–2.75 for a PAL of 1.55) were considered to be misreporters and were excluded from the analyses. Using EI:BMR from day 1 of the surveys maximised the sample because BMR was only provided for day 1 in the 1995NNS, whereas it was provided for both days in the 2012NNPAS. Sensitivity analyses were performed, where the data for all respondents including those considered to misreport were used (see Supporting information, Table S2). No differences in the findings were observed; however, misreported data were excluded from the reported analyses to provide more accurate estimates. The MULTIPLE SOURCE METHOD (MSM), version 1.0.1 [German Institute of Human Nutrition Potsdam-Rehbrücke (DIfE), Nuthetal, Germany] that was used to calculate usual intakes [38] in our previous analyses [39–41] mishandled the 1995NNS data and was considered unsuitable (data not shown).

Statistical analysis

Statistical analyses were performed using SPSS, version 21.0 (IBM Corp., Armonk, NY, USA). As a result of the large sample sizes of both surveys, a normal distribution was assumed and parametric tests were used [42]. Weighting factors were applied in accordance with the Australian Bureau of Statistics guidelines to extrapolate the results to the whole population. These factors account for the under- and/or over-sampling of particular categories of respondents and households that may occur as a result of the random nature of the sampling or non-responses [24]. Respondents were grouped by age and gender. For the sub-group analyses, the age groups related to those used by the Australian Dietary Guidelines [43] and Nutrient Reference Values [44]. Gender was considered binary as reported in each survey. Body mass index (BMI) (kg m^{-2}) was calculated and classified according to WHO categories [45].

To enable sub-group comparisons within each survey, total available carbohydrate, sugars, and added sugars data were converted into energy values (kJ) using Attwater factors [46]. Percentage contributions of each nutrient were calculated to account for the differences between the food composition data that were used in each survey. Total sugar and total added sugars were assumed to provide 16 kJ g^{-1}, whereas total carbohydrates provided 17 kJ g^{-1} in alignment with AUSNUT 2011–13 [24]. Differences in the reported intakes between genders and age groups were determined using one-way analysis of variance.

To collate the food group data, the highest percentage contributors to total added sugars for each survey were used and rank ordered. The mean percentage contributions of total added sugars from each food group were analysed according to both *per consumer* (main results) and *per capita* reported intakes (see Supporting information, Table S3).

To examine the contribution of the food groups to the inter-individual variation in total added sugar intakes, multiple linear regression models of the food group intakes (g) were created. Food group contributions to energy intake were used as an independent variable. Food groups that had less than 10 consumers were considered to be non-representative and excluded from the analyses. The regression coefficients represent the change in total added sugar intakes per 100-g increments in intake of the corresponding food group. The P values presented relate to the difference between the food groups reported in the 2012NNPAS (reference data) compared with the food group in the 1995NNS. $P < 0.001$ was considered statistically significant as a result of multiple comparisons.

Results

Data for 2148 of 10 851 adults in 1995NNS were considered implausible, with 1859 (17.1%) respondents considered extreme under-reporters and 289 (2.7%) respondents considered extreme over-reporters. A further 50 respondents were excluded from the analyses because they reported zero intakes of total added sugars, reducing the unweighted sample to 8653 (weighted $n = 8252$). Data for the 2012NNPAS ($n = 9341$) revealed 1593 respondents were considered to be extreme under-reporters and 124 respondents were considered to be extreme over-reporters, and were excluded from the analyses [47]. An additional 1318 respondent data did not include weight for the computation of EI:BMR and 38 respondent data reported zero intakes of total added sugars and thus these were excluded, reducing the unweighted sample to 6194 (weighted $n = 6289$) adult respondents (Figure 1).

Comparing the two surveys revealed no significant difference in the proportion of males and females who

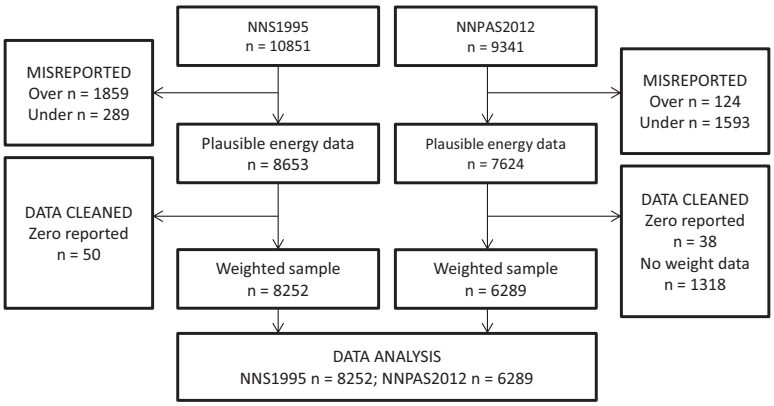

Figure 1 Flow diagram of included respondent data. 1995NNS, National Nutrition Survey 1995; 2012NNPAS, National Nutrition and Physical Activity Survey 2011-12.

Table 1 Demographic data and estimated nutrient intakes of respondents to the 1995NNS and 2012NNPAS data used for the total added sugars analyses*

Survey	1995 National Nutrition Survey				2012 National Nutrition and Physical Activity Survey				
Age group (years)	19-30	31-50	51-70	≥71	19-30	31-50	51-70	≥71	*P* value†
n	2087	3432	1935	798	1515	2333	1777	664	
Female (%)	45.1	46.5	46.5	56.0	43.1	47.3	48.7	52.6	
Mean BMI (kg m^{-2})	24.4 (4.1)	26.3 (4.6)	27.1 (4.3)	26.4 (3.9)	24.8 (4.7)	26.9 (5.0)	28.2 (5.1)	27.2 (4.7)	
Underweight (%)	3.7	1.0	0.8	1.3	4.3	1.4	1.2	1.4	<0.001
Healthy weight (%)	62.0	44.3	31.4	37.1	58.4	38.5	28.4	29.5	<0.001
Overweight (%)	25.6	38.5	46.7	44.6	26.9	38.8	40.4	44.7	<0.001
Obese (%)	8.7	16.2	21.1	17.0	10.4	21.4	30.0	24.4	<0.001
Total energy (kJ day^{-1})	11 724 (4534)	10 415 (3625)	9237 (2949)	7954 (2362)	10 848 (3624)	10 107 (3368)	9594 (3035)	8273 (2496)	<0.001
CHO (g day^{-1})	327.0 (133.5)	283.4 (109.8)	249.3 (89.7)	224.6 (72.9)	293.9 (113.7)	261.2 (103.6)	237.4 (98.3)	217.8 (81.2)	<0.001
CHO (%E)	48.0 (9.8)	46.7 (10.0)	46.4 (10.3)	48.4 (9.1)	46.4 (10.3)	44.3 (10.7)	42.3 (11.2)	44.8 (10.1)	<0.001
Total sugar (g day^{-1})	151.8 (84.3)	123.6 (69.7)	113.0 (58.5)	107.9 (50.4)	130.7 (73.7)	118.3 (68.2)	108.2 (62.6)	105.3 (53.5)	<0.001
Total sugar (%E)	21.0 (8.8)	19.1 (8.5)	19.8 (8.4)	21.9 (8.1)	19.4 (8.4)	18.9 (8.8)	18.1 (8.3)	20.3 (8.0)	<0.001
Total added sugars (g day^{-1})	89.7 (71.3)	63.7 (56.7)	49.1 (42.5)	45.9 (36.1)	77.7 (65.8)	63.3 (56.9)	52.5 (51.2)	49.9 (42.5)	<0.05
Added sugars to total sugar (%)	54.6 (23.7)	47.0 (23.3)	40.2 (21.3)	40.0 (19.7)	54.4 (24.8)	48.4 (23.9)	43.6 (23.1)	44.1 (21.0)	
Added sugar (%E)	12.2 (8.3)	9.6 (7.4)	8.4 (6.5)	9.1 (6.2)	11.3 (8.2)	9.9 (7.8)	8.5 (7.0)	9.4 (6.7)	

Nutrient data for day one of each survey shown as the mean (SD).

BMI data presented for *n* = 8209 1995NNS (*n* = 2087, 19–30 years; *n* = 3430, 31–50 years; *n* = 1928, 51–70 years; *n* = 764 ≥ 71 years); *n* = 6262 2012NNPAS (*n* = 1514, 19–30 years; *n* = 2329, 31–50 years; *n* = 1772, 51–70 years; *n* = 647 ≥ 71 years).

*Excludes pregnant women and respondents who misreported their intakes as estimated using Goldberg cut-offs. An additional 44 respondents in the 1995NNS and 37 in 2011-13NNPAS who did not have body mass index (BMI) data collected were excluded from BMI demographic data. Percentage values based in total survey data. Weighting factors have been applied to extrapolate result to the general Australian population. *P*-values for continuous variables tested by one-way analysis of variance. BMI categories based on World Health Organization classifications. CHO, carbohydrate; E, energy; 1995NNS, 1995 National Nutrition Survey; 2012NNPAS, 2011-12 National Nutrition and Physical Activity Survey.

†*P*-values for continuous variables tested by one-way ANOVA, *P*-values for categorical variables tested by Pearson's X^2.

reported their dietary intakes ($P = 0.442$). There was a significant difference between the two surveys by age group, with a larger proportion of respondents aged 31–50 years in these 1995NNS data (41.6% compared to 37.1% 2012NNPAS) ($P < 0.001$) and a higher number of persons born in English speaking countries, including Australia, in these 1995NNS data (73.9% versus 69.2% 2012 NNPAS) ($P < 0.001$). The mean (SD) BMI for respondents aged over 18 years fell within the overweight category for both surveys [1995NNS 25.99 (4.45) kg m^{-2}, 2012 NNPAS 26.78 (5.06) kg m^{-2}, $P < 0.001$]. Total added sugar intakes decreased from 62.1 (54.4) g day^{-1} in the 1995NNS to 60.0 (54.0) g day^{-1} in the 2012NNPAS ($P = 0.018$). Added sugar intakes contributed to 20.1 (8.6%) and 19.0 (8.5%) of the total sugar intakes for adult respondents in the 1995NNS and 2012NNPAS, respectively, and represented 10.8% 1995NNS and 10.6% 2012NNPAS of total reported energy intakes (Table 1).

In total, 24 food groups were identified for consumers of food groups that contributed added sugars (Table 2). These food groups differed compared to the data for all respondents (per capita) (see Supporting information, Table S3) and also demonstrated greater variability in the

Table 2 Top 20 food group contributors to total added sugars intake in the 1995NNS and 2012 NNPAS

	1995NNS				2012NNPAS				
Food groups	Rank	*n*	Mean	SD	Rank	*n*	Mean	SD	*P* value
Sweetened beverages	1	2768	45.8	30.2	2	2251	35.6	31.8	<0.001
Sugar, honey and syrups	2	5387	36.6	27.4	4	3049	32.4	25.7	<0.001
Dishes and products other than confectionery where sugar is the major component	3	224	29.5	23.2	9	161	26.9	22.9	0.277
Chocolate and chocolate-based confectionery	4	1319	29.4	22.8	6	1147	31.6	25.3	0.023
Other alcoholic beverages	5	197	28.8	27.6	5	287	31.7	30.6	0.297
Flavoured milks	6	365	27.9	23.9	3	315	34.7	25.2	<0.001
Cake-type desserts	7	2232	27.8	23.8	1	1152	42.7	25.9	<0.001
Milk and milk products based dishes	8	688	25.6	19.9	25	260	9.5	18.4	<0.001
Frozen milk products	9	1478	25.5	20.7	7	918	29.6	22.6	<0.001
Sweet biscuits	10	2352	21.4	20.7	12	1467	21.7	20.1	0.582
Other confectionery	11	605	21.2	19.0	11	636	25.3	23.4	<0.001
Fruit dishes	12	70	18.8	21.8	8	39	29.3	21.6	0.017
Milk substitutes	13	210	18.0	21.5	13	267	19.7	24.4	0.440
Canned condensed soup	14	24	17.7	31.1	30	132	6.0	13.7	0.003
Cereal-, fruit-, nut-, and seed-bars	15	234	17.1	18.2	14	420	17.4	18.4	0.856
Fruit and vegetable juices and drinks	16	3300	17.1	25.7	17	1605	16.7	25.3	0.609
Formula dietary foods	17	53	16.7	22.2	20	205	12.2	21.8	0.187
Batter-based products	18	353	15.1	18.4	24	245	10.3	16.2	<0.001
Yogurt	19	757	13.7	20.8	18	1073	15.7	20.4	0.037
Jam and lemon spreads, chocolate spreads	20	1729	13.4	17.7	10	666	26.3	23.5	<0.001
Pastries	21	1619	9.4	17.4	22	984	10.8	18.3	0.054
Pickles, chutneys and relishes	22	959	8.0	14.8	26	369	9.3	16.6	0.139
Breakfast cereals	23	3748	7.64	17.43	16	2309	16.9	21.8	<0.001
Gravies and savoury sauces	24	3429	7.4	15.3	23	1628	10.5	18.3	<0.001
Other beverages	25	774	6.4	12.0	15	472	16.9	21.0	<0.001
Pretzels and other snacks	26	34	6.1	12.9	45	56	2.2	5.9	0.052
Mixed dishes with fish or seafood	27	227	5.9	14.7	42	131	2.5	7.4	0.015
Cereal-based dishes	28	1718	5.6	13.0	27	2225	8.3	16.7	<0.001
Salad dressings	29	1490	5.4	11.3	29	1186	6.8	14.5	0.004
Stone fruit	30	800	4.8	13.8	34	587	4.0	12.8	0.291
Savory biscuits	31	1322	4.5	12.6	33	1000	4.9	14.2	0.530
Fancy breads	32	1120	3.9	11.8	37	827	2.9	10.2	0.045
Corn snacks	33	187	3.9	15.4	40	162	2.6	9.0	0.350
Soup	34	1086	3.4	10.0	39	423	2.7	9.2	0.236
Artificial sweetening agents	35	583	3.3	14.4	47	303	1.4	7.4	0.034
Fruit combinations	36	231	2.8	9.0	19	251	14.3	17.8	<0.001

Data shown as the mean (SD) per consumer contribution to total added sugars intake. Food group rankings bolded for the top 20 groups.
1995NNS, 1995 National Nutrition Survey; 2012NNPAS, 2011–12 National Nutrition and Physical Activity Survey.

food group ranking for their contributions to total added sugars between the surveys. Nine of the top 20 food groups from the 1995NNS had significant differences in their added sugars contribution between the 1995NNS and 2012NNPAS. Three food groups ('alcoholic beverages' ranked fifth, 'other confectionery' ranked eleventh and [dairy] 'milk substitutes' ranked thirteenth) did not differ in their rankings of added sugars contribution between the 1995NNS and the 2012 NNPAS. The largest shifts (>25 places) in the food group contributions to added sugars were seen for 'mature legume and pulse dishes' (37/88 places), with 'hot porridge' (28/88 places) ranking higher in the 2012 NNPAS and 'other vegetables and combinations' (34/88 places) ranking higher in the 1995NNS.

For the sub-analysis by gender, the top two food group contributors to added sugars were found to be 'sweetened beverages' and 'sugars, honey and syrups' for the 1995 NNS, with 'cake type desserts' moving into the highest ranking for the 2012NNPAS with increased variability in the other food group contributors (Table 3). When analysing these data by age category, fewer similarities were seen for the 31–50 year old group, with no common rankings of food groups being found between the surveys. The top three food group contributions by age also varied. 'Sweetened beverages' (40%–50% added sugar intake) remained top ranked up to the age of 70 years for the 1995NNS, shifting to also include 'cake-type desserts' and 'fruit dishes' in the 2012NNPAS (see Supporting information, Table S4).

The main sources of added sugars for all adult respondents (per capita) did not vary substantially with more than 40% of the added sugars contribution (51.67% 1995NNS, 42.46% 2012NNPAS) coming from the same four food groups ('sugar, honey and syrups', 'sweetened beverages', 'breakfast cereals (read-to-eat)' and 'cake-type desserts') across the two surveys (see Supporting information, Table S2). However, there were significant differences in the contributions to the first three food groups ($P < 0.001$). The largest change in food group rankings between 1995NNS and 2012NNNPAS was for 'other vegetables and combinations', which moved by down by 60 places ($P < 0.001$). The age-stratified analyses demonstrated greater differences in the top three food group contributions to added sugars between the per consumer and per capita analyses (see Supporting information, Table S5). These analyses also showed substantial variations in the number of respondents consuming each food group. For example, although the top ranked food group was consumed by only 2786 respondents from the 1995NNS, the second ranked food group was consumed by almost double the number of respondents (5387 respondents), providing insights into the quantities of 'sweetened beverages' that were reported at the time of the survey.

The food group contributions to total added sugars intake by age group showed that the 'sugar, honeys and syrups' and the 'sweetened beverages' food groups were the top contributors between the surveys up to the age group of 70 years. 'Fruit and vegetable juices and drinks', 'chocolate based confectionery', 'cake-type desserts' and 'sweet biscuits' food groups provided varying contributions across all of the age categories. Only the 'sugars, honeys and syrups' food group contributed to >10% of the total added sugars across all age categories and contributed to >20% for respondents aged 71 years and over for both surveys ($P < 0.001$) (see Supporting information, Table S4).

The top food groups contributing to total added sugars were similar by gender for each survey, with some variability in the food group rankings beyond the two top food groups of 'sugar, honey and syrups' (male $P < 0.001$, female $P < 0.001$) and 'sweetened beverages' (male $P = 0.025$, female $P < 0.001$) (Table 4 and Supporting information, Figures S1 and S2). This was reflected in the model of inter-individual variation, where the 'sugar, honey and syrups' ($\beta = 83.70$, $P < 0.001$), 'chocolate and chocolate-based confectionery' ($\beta = 44.30$, $P < 0.001$), and 'other confectionery' (β: 61.40, $P < 0.001$) food groups were significant predictors of total added sugar intakes for these 1995NNS ($r^2 = 0.755$) data. 'Sugar, honey and syrups' ($\beta = 88.75$, $P < 0.001$), 'jam and lemon spreads chocolate spreads' ($\beta = 45.82$, $P < 0.001$) and 'chocolate and chocolate-based confectionery' ($\beta = 40.79$, $P < 0.001$) food groups were significant predictors of total added sugars intake for these 2012NNPAS ($r^2 = 0.740$) data (Table 4).

Discussion

The present study reports a minimal decrease in the intake of added sugars across two nationally representative Australian nutrition surveys conducted 16 years apart with differences in the main contributing food groups, particularly when analysed by intake of consumers only. Our results are consistent with previous studies [16,17] over the same time period, although those analyses only reported per capita food group contributions.

The difference in the proportion of energy from added sugar was most prominent for respondents aged 71 years and over, which is comparable to another study with older Australians [28]. Despite this, the only age group that did not meet the WHO [21] target of <10% of energy across both surveys comprised those aged 19–30 years. The decrease in added sugars over time in this age group was encouraging, although may be even further from the target than our results may demonstrate. Our definition of added sugars differed from the WHO definition of free sugars that includes fruit juice. This meant that, for the

Table 3 Gender stratified food group contributions to total added sugars intake for adult respondents showing top 20 food group contributors in the 1995NNS and 2012NNPAS by the 20 highest contributors of AS in 1995NNS and 2012NNPAS, stratified by gender

	Male									Females								
	NNS				NNPAS					NNS				NNPAS				
Food Group	*n*	rank	Mean	SD	*n*	rank	Mean	SD	*P*	*n*	rank	Mean	SD	*n*	rank	Mean	SD	*P*
Sweetened beverages	1584	1	47.8	28.9	1368	2	38.7	31.2	<0.001	1049	1	42.8	31.9	883	8	30.6	32.0	<0.001
Sugar, honey and syrups	2946	2	37.4	27.7	1658	4	31.3	25.0	<0.001	2177	2	35.5	27.1	1392	5	33.8	26.5	0.068
Dishes and products other than confectionery where sugar is the major component	85	3	28.5	23.5	77	9	25.5	22.5	0.410	128	5	30.1	23.0	85	9	28.2	23.3	0.542
Flavoured milks	226	4	28.1	24.3	204	3	33.6	23.3	0.018	120	8	27.6	23.1	112	3	36.8	28.4	0.007
Other alcoholic beverages	71	5	27.5	28.4	153	5	30.0	30.2	0.558	117	6	29.6	27.2	134	6	33.5	31.1	0.292
Chocolate and chocolate-based confectionery	612	6	26.4	20.5	546	8	27.9	24.1	0.247	642	3	32.3	24.4	601	4	35.0	26.0	0.060
Milk and milk products based dishes	306	7	24.1	18.8	132	28	7.6	16.5	<0.001	348	9	26.9	20.7	128	24	11.6	20.0	<0.001
Frozen milk products	801	8	23.5	18.5	527	6	28.2	21.9	<0.001	604	7	28.2	23.0	391	7	31.3	23.6	0.039
Cake-type desserts	996	9	23.5	20.6	544	1	39.0	23.7	<0.001	1126	4	31.7	25.7	608	1	46.0	27.3	<0.001
Canned condensed soup	10	10	21.6	30.0	74	27	7.8	16.1	0.026	13	18	14.5	32.9	58	33	3.7	9.6	0.035
Fruit dishes	38	11	20.0	25.7	26	10	25.2	19.0	0.391	29	15	17.3	15.9	13	2	37.7	24.7	0.002
Sweet biscuits	1137	12	20.0	19.2	747	12	21.4	20.0	0.110	1099	11	22.8	22.0	721	12	22.0	20.1	0.458
Other confectionery	255	13	19.2	18.1	280	11	22.3	21.4	0.075	320	10	22.8	19.5	356	10	27.7	24.7	0.005
Fruit and vegetable juices and drinks	1605	14	18.9	26.5	893	17	16.2	24.7	0.011	1532	17	15.2	24.8	712	17	17.4	26.0	0.056
Cereal-, fruit-, nut-, and seed-bars	112	15	16.6	19.2	238	15	16.5	19.0	0.959	110	14	17.7	17.3	182	15	18.6	17.6	0.663
Milk substitutes	88	16	15.0	19.2	105	13	20.7	26.9	0.096	112	13	20.4	23.0	162	14	19.0	22.7	0.620
Batter-based products	178	17	13.7	15.4	130	20	11.7	18.3	0.300	157	16	16.7	21.2	114	27	8.6	13.1	<0.001
Jam and lemon spreads, chocolate spreads	856	18	12.8	16.5	338	7	28.0	25.0	<0.001	788	20	14.0	18.8	328	11	24.5	21.7	<0.001
Formula dietary foods	28	19	12.6	17.9	127	26	7.9	15.4	0.163	22	12	21.9	26.3	78	13	19.2	28.2	0.694
Yogurt	269	20	12.6	17.9	429	19	14.1	18.6	0.289	450	19	14.3	22.3	644	19	16.8	21.5	0.066

1995NNS, 1995 National Nutrition Survey; 2012NNPAS, 2011–12 National Nutrition and Physical Activity Survey.

Calculated as per consumer mean (SD) contribution to total added sugar intakes. Added sugars definition used in this study excluded fruit and fruit juices. Fruit and vegetable juices and drinks categories are included in food group analyses to demonstrate their contribution of other sugars.

Table 4 The top 20 food groups for total added sugars showing inter-individual variations in the 1995NNS and 2012NNPAS

Food group	β	SE	Partial r^2	*P* value
1995 National Nutrition Survey[†]				
Sugar, honey and syrups	83.70	1.30	0.293	0.000
Sweetened beverages	8.00	0.10	0.511	0.000
Cake-type desserts	12.80	0.40	0.073	0.000
Juices and drinks	5.10	0.10	0.172	0.000
Sweet biscuits	25.10	1.30	0.035	0.000
Pasta products	16.50	1.00	0.023	0.000
Chocolate confectionery	44.30	1.30	0.104	0.000
Frozen milk products	12.10	0.50	0.054	0.000
Gravies and savoury sauces	0.40	0.50	0.000	0.448
Breakfast cereals (ready-to-eat)	7.90	0.70	0.012	0.000
Jam and sweet spreads	16.60	2.80	0.003	0.000
Milk and milk products based dishes	7.00	0.50	0.017	0.000
Pastries	0.50	0.30	0.000	0.092
Other confectionery	61.40	2.40	0.060	0.000
Flavoured milks	3.50	0.20	0.019	0.000
Yogurt	0.50	0.50	0.000	0.361
Cereal-based dishes	0.30	0.20	0.000	0.159
Salad dressings	−2.90	2.60	0.000	0.257
Pickles, chutneys and relishes	7.10	2.30	0.001	0.002
Savoury biscuits	0.70	2.00	0.000	0.721
2012 National Nutrition and Physical Activity Survey[‡]				
Sugar, honey and syrups	88.75	1.83	0.225	<0.001
Other confectionery	56.50	1.95	0.093	<0.001
Jam and lemon spreads, chocolate spreads	45.82	2.28	0.047	<0.001
Chocolate and chocolate-based confectionery	40.79	1.19	0.127	<0.001
Sweet biscuits	29.06	1.29	0.058	<0.001
Cake-type desserts	23.82	0.51	0.211	<0.001
Frozen milk products	13.79	0.56	0.070	<0.001
Cereal-, fruit-, nut-, and seed-bars	8.61	2.29	0.002	<0.001
Sweetened beverages	6.87	0.08	0.488	<0.001
Salad dressings	6.45	2.57	0.001	0.012
Other alcoholic beverages	6.06	0.19	0.114	<0.001
Flavoured milks	5.39	0.21	0.073	<0.001
Other beverage flavourings and prepared beverages	4.13	0.55	0.007	<0.001
Fruit and vegetable juices and drinks	3.97	0.14	0.086	<0.001
Breakfast cereals (ready-to-eat)	3.54	0.77	0.003	<0.001
Yogurt	2.78	0.41	0.006	<0.001
Milk substitutes	2.42	0.58	0.002	<0.001
Gravies and savoury sauces	2.22	0.67	0.001	0.001
Pastries	0.96	0.40	0.001	0.015
Cereal-based dishes	−0.13	0.15	0.000	0.397

1995NNS, 1995National Nutrition Survey; 2012NNPAS, 2011–12 National Nutrition and Physical Activity Survey.
[†]Model r^2 = 0.755.
[‡]Model r^2 = 0.740.

food group contributions, 'fruit and vegetable juices and drinks' were the third ranked contributor to added sugars for both surveys for this age group. At present, the Australian Food Standards Code provides an upper limit of the total sugars composition or all fruit juices [48], whereas fruit drinks are permitted to contain sugars with no upper limit specified [49]. Our analyses demonstrate the large contribution that these beverages can provide within the diet from sugars added during manufacturing or processing.

The main food group contributor to total added sugars was 'sugar, honey and syrups' and this remained consistent over time [50]. Similar findings were reported in another study that used the same added sugars method as that employed in the present study [28]. Shifting food group contributions to total added sugars reflect changes

in the food supply between 1995 and 2012. For example, a decline in the added sugars contribution from 'cereal–based dishes', 'mature legume and legume based dishes' (including baked beans) and 'milk and milk product based dishes' suggests that food reformulations may have occurred over time. A similar shift was seen in Australia for sodium, where product reformulation has occurred across a range of processed food categories [51,52]. Another interesting shift that emerged from our analyses was the movement of 'cake type desserts' to the first ranked food group contributor in the NNPAS2012 per consumer analysis. This shift in ranking suggests that there has been a change in eating patterns for cakes likely supported by the growing 'coffee culture' that was not as apparent in Australian society during the 1990s [53].

The method of analysis used in the present study also exposes the number of consumers for each food group and the difference in such numbers between the surveys and between the more commonly used per capita analyses. Because added sugars are consumed across a wide range of food groups, this type of analysis also begins to separate out the more frequent from the less frequently consumed food groups and provides added insight into the amount of particular food groups that are being consumed. By comparison with 'cake-type desserts', more consumers reported consuming 'sweet biscuits', yet this only ranked in twelfth place in the same survey as a result of their smaller quantities. Furthermore, if the contribution of added sugars per food group were expressed as a ratio per consumer (data not shown), then this would increase the ranking of the 'fruit dishes' food group, which was consumed by only 39 respondents. Such changes in intake demonstrate the differences in consumer eating patterns, where certain food groups are reportedly consumed more often and others are considered to be episodically consumed and warrant further exploration. Such exploration may require the need for usual intake adjustment, as has previously been explored by our team when looking at Australian meat intakes [54]. This more robust usual intake model should be explored for the 2012NNPAS added sugars data that has revealed a different pattern of eating, likely snacking from this study. Added sugars intake also appear to demonstrate greater variation amongst the respondents by comparison to meat. This may reflect added sugars as components of a food rather than a food type.

For dietary guidance and food policy compliance, it is important to identify the food groups contributing to added sugars intake. In Australia, dietary guidelines provide qualitative rather than quantitative recommendations to consume added sugars 'only occasionally and in small amounts' [55] and, for the purpose of nutrition education, foods containing added sugars are considered to be 'discretionary' foods. Despite such guidance, key food sources of added sugars intake across the population have remained consistent between the two surveys. 'Sugar, honey and syrups' and 'sweetened beverages' are two discretionary food groups that have remained the largest contributors to both male and female intakes up to the age of 70 years. Percentage contributions to added sugars for the top three food groups also comprised >40% of total added sugars for all age groups, reaching >50% for those aged 19–50 years. This was noteworthy in the age-stratified analyses, where a change in 'sweetened beverage' contributions to added sugars was seen for the analysis by all respondents but not compared to the per consumer analysis. Discretionary food sources were again identified in those analyses considering the inter-individual variability in the surveys. Three of the main food group predictors remained consistent between the surveys, yet they differed in their food group rankings both by age and gender category. This change to the predictive foods models may relate to shifts in the eating patterns between 1995 and 2012. Although 'sugar, honeys and syrups' remained the main predictor, this food group is almost entirely consistent of sugar as the primary ingredient. The contribution from 'chocolate and chocolate based confectionery' decreased over time, which is likely to reflect a wider range of food product availability. These products have also undergone a decrease in manufacturer serving size over time which may have impacted this shift.

Regardless of the differences in the food groupings, our findings are in agreement with previous reports from the individual surveys. Cobiac *et al.* [18] found 'non-alcoholic beverages' to be the biggest contributors in 1995NNS (encompassing both juice-based drinks and sugar based drinks including soft drinks, sports drinks, flavoured mineral water and cordials), whereas Lei *et al.* [17] reported that 'sugar sweetened beverages' (including fruit drinks, cordials/mixers, soft drinks, energy drinks and other sweetened beverages) were the main sources in 2012NNPAS. A longitudinal meta-analysis of added sugars intake from adolescence to early adulthood also found sugar sweetened beverages to be strong contributors, although a decrease with increased age was noted. The meta-analysis looked at multiple metrics of intake for participants and, based on servings per week, found a significant decrease in confectionery (−0.20 servings) consumption and a non-significant decrease in sugar sweetened beverage (−0.15 servings) consumption, as well as an annual decrease (−0.15% total energy) in added sugars or sucrose consumption [56]. A similar pattern was seen in the present study, where the amount of added sugars consumed from 'sweetened beverages' also decreased with increasing age up until 70 years.

Targeting sugar sweetened beverages as a strategy to reduce overall added sugar intake has had mixed success

globally. The WHO suggested a minimum 20% tax be added to sweetened beverages to reduce sales and, in turn, reduce associated obesity rates [57]. A tax in Mexico has been associated with a mean 7.6% decline in purchase rates, paralleled by a 2% increase in non-taxed beverages [58]. Other countries have previously introduced taxes on foods and beverages, although it may be argued that these measures are not likely to be effective in changing behaviours if the taxes are not sufficiently high, as shown in some parts of the USA [59]. The UK introduced a sugar tax levy in April 2018 where manufacturers of products containing greater than 8 g of sugars per 100 mL were charged. Analyses of impact have demonstrated that the added costs have been passed on to the consumer in categories outside of the intended targets, although product reformulation has also been occurring [60]. A model of the impact for Australia has shown that a similar tax would substantially impact body weight and the associated health costs [61], although there is a lack of political mandate to the introduction of a tax on sweetened beverages.

A strength of the present study was the use of consistent methodology to assess added sugars intake using the two surveys. This has allowed comparisons that were previously not possible as a result of differences in the databases used to analyse each survey. The 1995NNS also included total carbohydrate in its analysis, whereas, in 2012NNPAS, this was replaced with available carbohydrate, which excludes fibre and carbohydrates that cannot be digested [24,62,63]. Our analyses applied the relevant Atwater factors to allow for comparability. There are, however, some limitations to the present study. These intake data used for both surveys were obtained via 24-h recalls that relied on self-reporting and the memory of the respondents [64]. The 24-h recall methodology only captures information about the previous 24-h period of eating and cannot be considered to represent usual intake. Although an attempt was made to model the data to usual intake values using the MULTIPLE SOURCE METHOD (MSM) and 2 days of reported 24-h recalls, the model mishandled the 1995 data and was unsuccessful. Thus, the resultant values used are for one day of reporting only. Previous studies from our team have shown that, for commonly consumed foods, such as the sources of added sugars, the difference resulting from using data from day 1 and a usual intake model is minimal [54]. Simulation studies have also shown that, with large sample sizes, such as those used in the present study, the differences between the intake models become less apparent [65]. Furthermore, misreporting is inherent to the 24-h recall methodology and often occurs with incorrect estimations of portion sizes and the omission of condiments or whole food items. Although the study adjusted for misreporters, we also undertook analyses for age and gender in an attempt to limit the impact of the changes in the response patterns and reported energy intakes between the two surveys. In addition, the sensitivity analyses demonstrated no difference in the direction of change in added sugar intakes when excluding misreporting respondents. Our analysis also included a 'per consumer' approach aiming to better understand how the different food group contributors are consumed. Using only one day of intake data has limitations with respect to assessing the potential impact of 'snacking', which may be highly variable from day to day and also be of relevance when considering foods that are high in added sugars.

Despite using nationally representative surveys of Australian adults, there were differences in the recruitment strategies between surveys. The 1995NNS respondents were a subsample of volunteers recruited from the 1995 National Health Survey, whereas the 2012NNPAS respondents were a random a subsample of the AHS [24]. In addition, the 1995NNS included individuals from very remote areas, which may impact on the generalisability and comparability of the surveys. The methods of administration of the 24-h recall also differed between the surveys, with one using a food portion guide that may affect the accuracy of the amount of food recalled, although this should not have an effect on recall of food types, specifically [66].

In conclusion, a comparison of Australian dietary intake survey data between 1995NNS and 2012NNPAS has indicated consistent sources of the main contributions to added sugars for the population as a whole. Although the intakes did vary by gender and age group, discretionary (occasional) food sources were the key contributors. Because food patterns vary largely between consumers, our novel analysis of intakes by food group consumers for added sugars between 1995 and 2012 did demonstrates change over time. These changes may be affected by dietary trends including the low or sugar free diets that have become increasingly popular [67]. In addition to addressing consumer changes over time, further research is needed to determine the impact of consuming added sugars on the potential displacement of other food groups within the diet. Such analyses may also allow researchers to consider the impact of added sugars on chronic disease risk factors. In turn, information about the relationships between particular categories of added sugars food sources may be associated with health risk such as obesity in later life. Our findings also support the need for public health campaigns to target whole food sources of a nutrient matrix rather than focussing on single nutrients in isolation [11]. Overall, 'sweetened beverages' remain one of the largest contributors to added sugars consumption, with changing eating patterns seeing

'cake type desserts' being more regularly consumed. These food sources represent a potential target for the public health strategies.

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Conflict of interests, source of funding and authorship

The authors declare a conflict of interest for YP, KC and JCYL together, who jointly supervised a PhD candidate with a focus on added sugars, as well as for JCYL alone, who consults with the Glycaemic Index Foundation.
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YP, KC and JCYL designed the research. SD conducted the research. YP, SD and JCYL analysed data and performed the statistical analyses. YP, SD, KC and LCYL performed the data interpretation. YP, KC, JCYL, SD drafted the manuscript. YP and KC had primary responsibility for the final content and approved the final manuscript. All authors critically reviewed the manuscript and approved the final version submitted for publication.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with STROBE guidelines. The lead author affirms that no important aspects of the study have been omitted.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Percentage contribution of added sugars to total sugars by food group for adult male survey respondents

Figure S2. Percentage contribution of added sugars to total sugars by food group for adult female survey respondents

Table S1. Re-coding of food groups in the AUSNUT food composition databases.

Table S2. Food group contributions (%) to total added sugar intakes for adult respondents *per capita* in 1995NNS and 2012NNPAS showing the top 20 food groups.

Table S3. Age stratified *per consumer* food group contributions (%) to total added sugar intakes for adult respondents showing changes in the ranking of the top 20 food groups.

Table S4. Age stratified *per capita* food group contributions (%) to total added sugar intakes for adult respondents showing changes in the ranking of the top 20 food groups in 1995NNS and 2012NNPAS.

Table S5. Mean (SD) percentage contribution (%) to total AS intake by the 20 highest contributors of AS in 1995NNS and 2012NNPAS, all respondents included (sensitivity analysis).

BEVERAGES

Prevalence and pattern of energy drink intake among Australian adolescents

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Abstract

Background: Energy drinks (ED) are popular among young people despite evidence of associated health risks. Research into the prevalence and pattern of ED intake among young people is sparse. The present study investigates the prevalence and pattern of ED intake among a large sample of adolescents, including how many consume them, how often, for what reasons and in what contexts.

Methods: In 2018, all students in grades 7–12 attending 25 randomly selected Western Australian schools were invited to complete an online self-report survey about EDs.

Results: Of the 3688 respondents, 51.2% reported consuming an ED. Of these 'ever consumers', 23.4% drank them monthly, 19.2% weekly and 2% every day. The average age of first intake was 10.7 years. One-fifth (19.7%) of 'ever consumers' reported consuming more than two EDs in 1 day. Reasons for ED use included taste, to boost energy levels, sport performance and studying.

Conclusions: The findings add to limited international evidence about adolescent ED use and provide valuable information to help ensure interventions to reduce intake address the underlying reasons and contexts of ED consumption.

Introduction

Energy drinks (EDs) are flavoured, non-alcoholic beverages that contain caffeine and are marketed to improve mental and/or physical performance [(1)]. Often brightly coloured and attractively packaged, EDs have catapulted to popularity among young people and have become one of the fastest growing segments of the beverage market. Although comparable to soft drinks in sugar and energy content, EDs contain, on average, around three or more times the amount of caffeine (around 160 mg per 500 mL in Australia, which is equivalent to two cups of instant coffee). Other common ingredients include amino acids, herbal stimulants and sodium, which may interact with caffeine and exacerbate its effects [(2)].

Energy drinks have been associated with a wide range of adverse health outcomes. For example, two recent systematic reviews of serious adverse events occurring following consumption of EDs have linked EDs with a number of cardiovascular and neurological problems,

including arrhythmias, myocardial ischaemia, aneurysm/dissection, cardiac arrest, vasospasm, coronary thrombosis, cardiomyopathies, hypertension, seizures, cerebrovascular accident and neuro-psychiatric events (suicidal ideation and psychosis) [2,3]. There is also evidence linking ED intake in children, adolescents and young adults with higher rates of smoking, alcohol (including binge-drinking) and other substance use, sensation seeking, self-destructive behaviour, problems with behavioural regulation and metacognitive skills, increased sedentary behaviour, headaches, stomach aches, hyperactivity and sleeping problems, insomnia, tiredness/fatigue, irritation, and hyperactivity/inattention symptoms [4]. Concern over the health effects associated with these drinks has prompted several countries to ban the sale of these drinks to people aged under 18 years, or altogether.

A recent review into the consumption of EDs by young people noted that, despite the growing ED market and reports of serious adverse events associated with their consumption, research into ED use was sparse, with most studies being conducted in Europe or North America [4]. For example, in 2011, the European Food Safety Authority (EFSA) commissioned a study to gather ED consumption data on over 52 000 people within 16 countries of the European Union and found adolescents (aged 10–18 years) had the highest prevalence of consumption (68%) followed by adults (18–65 years, 30%) and children (3–10 years, 18%) [5]. Thus, consideration of the patterns and reasons for ED use and non-use among adolescents (a key ED consumer group) may help inform future interventions.

The present study aimed to investigate quantitatively the prevalence and pattern of ED intake among a large sample of Australian adolescents, including how many consume them, which brands are commonly consumed, how often, for what reasons and in what contexts. The reasons why adolescent non-ED users choose not to drink EDs were also explored.

Materials and methods

School selection and participants

Non-government (n = 30) and government (n = 65) Western Australian schools were randomly selected based on their school Index of Community Socio-Educational Advantage (ICSEA) score [6] to ensure representation across socio-economic status. School principals were contacted and invited to participate in the 'AMPED UP: An Energy Drink Study', which involved an online survey completed by secondary school students during class time. Of the 95 schools contacted, 25 (26%) agreed to participate.

All grade 7–12 students in participating schools were invited to take part (except for three schools only able to invite grades 7–10). Student participation via active parental consent was 27%, ranging from 10%–66% of the entire school.

Amped up survey

Survey questions were adapted from the 2015 Canadian Adolescent and Young Adult Energy Drink Survey by Hammond *et al.*[7] and included socio-demographics and a range of items to measure prevalence, pattern, context and reasons for ED intake and non-ED intake.

Ethics

Ethics approval was obtained from The University of Western Australia's Human Research Ethics Committee, The WA Department of Education, The WA Catholic Education Office and the Association for Independent Schools of WA. Active informed consent was obtained from each school principal as well as each participating child and their parent/guardian.

Statistical analysis

Of the 3837 surveys received, 3688 met minimal data requirements (i.e. contained data on ED intake). Descriptive statistics were generated in SPSS, version 25 (IBM Corp., Armonk, NY, USA) and were used to answer the research aim.

Results

Participant characteristics and prevalence of ED consumption is shown in Table 1.

The top five ED brands preferred by participants included 'Red Bull™' (Red Bull GmbH, Fuschl, Austria) (n = 544), 'Monster™' (Monster Energy Company, Warriewood, NSW, Australia) (n = 372), 'Mother™' (Coca-Cola Amatil (Aust) Pty Ltd, Northmead, NSW, Australia) (n = 309), 'V™' (Frucor Suntory Australia Pty Ltd, North Strathfield, NSW, Australia) (n = 289) and 'Rockstar™' (Frucor Beverages (Australia) Pty Ltd, North Strathfield, NSW, Australia) (n = 212) (all these ED brands contain a similar caffeine content). EDs were more likely to be consumed on weekends (69%) than weekdays (31%). The most common time of day for ED consumption was in the afternoon (13.30 h to 17.30 h). Fifteen percent of ED 'ever consumers' (defined as ever tried an ED, even a few sips) reported 'always' or 'usually' consuming a sugar-free or low-energy ED. One quarter of ED 'ever-consumers' reported having consumed a 710-mL ED can.

The reasons and context for ED-use and non-use are shown in Figure 1.

Table 1 Participant characteristics and prevalence of energy drink (ED) intake (n = 3688)

Characteristic	Count	%
Sex[a]		
Female	2032	55.1
Male	1655	44.9
Age, mean (SD)	3688	13.6 (1.5)
Grade		
Grade 7	1023	27.7
Grade 8	865	23.5
Grade 9	732	19.8
Grade 10	605	16.4
Grade 11	290	7.9
Grade 12	173	4.7
School socio-economic status		
High	1854	50.3
Low	1834	49.7
School location		
Metropolitan	2849	77.3
Regional	839	22.7
Has tried an ED		
Yes	1889	51.2
No	1799	48.8
Frequency of ED intake		
Whole sample[b]		
Never	1799	50.6
Rarely/<1 per month	1009	28.4
Monthly	410	11.5
Weekly or more	338	9.5
Lower school (grades 7–8)[c]		
Never	1000	55.2
Rarely/<1 per month	418	23.1
Monthly	205	11.3
Weekly or more	190	10.5
Middle school (grades 9–10)[d]		
Never	614	47.4
Rarely/<1 per month	413	31.9
Monthly	154	11.9
Weekly or more	115	8.9
Upper school (grades 11–12)[e]		
Never	185	41.4
Rarely/<1 per month	178	39.8
Monthly	51	11.4
Weekly or more	33	7.4
Males[f]		
Never	707	44.4
Rarely/<1 per month	469	29.4
Monthly	221	13.9
Weekly or more	196	12.3
Females[g]		
Never	1092	55.7
Rarely/<1 per month	540	27.5
Monthly	189	9.6
Weekly or more	141	7.2
ED 'ever-consumers' (n = 1889)*		
Frequency of ED intake[b]		
Rarely/<1 per month	1009	57.4
Monthly	410	23.4
Weekly	302	17.2
Everyday	36	2.0
Age first ED consumed, mean (SD)[h]	1885	(10.73, 2.93)
Largest number of EDs consumed in one day[i]		
Don't know	270	15.8
1	788	46.2
2	312	18.3
3	153	9.0
4	64	3.8
5 or more	117	6.9

Missing responses: [a]n = 1; [b]n = 132; [c]n = 75; [d]n = 41; [e]n = 16; [f]n = 62; [g]n = 70; [h]n = 4; [i]n = 185.

*Participants were classified as an ED 'ever consumer' if they responded affirmatively to the question, 'Have you ever tried an energy drink, even a few sips?'

Discussion

The present study found a high prevalence of ED intake among Australian adolescents, with half the sample (49.4%) reporting trying an ED and one in 10 (9.5%) drinking them weekly or more. Of those who tried an ED, 19.7% consumed more than two in 1 day and the average age of first ED consumption was 10.7 years. ED intake was more frequent among males and older age groups. These results are consistent with previous Australian [8] and North American [9,10] studies, which have reported similar findings in terms of lifetime prevalence among adolescents, initial age of consumption, and gender and age differences, although less than the 68% average lifetime ED prevalence reported in the EFSA study involving 16 European countries [5].

In terms of reasons for and context of ED use, our results support previous findings that adolescents like the taste of EDs use them to feel more energetic, awake and/or alert, boost sports performance and help them study [11,12]. We also found parents and the home environment were key factors associated with the reasons and context for adolescent ED intake; home was the place EDs were consumed most-often, and one-fifth of ED users reported obtaining EDs from a parent. Conversely, 74% of non-ED users reported they did not drink EDs because they were advised not to by their family. In addition to the home, supermarkets/grocery-stores, friends, petrol (gas) stations and convenience stores were among the most common places adolescents in our sample obtained EDs.

Overall, our results suggest that health promotion programmes aimed at reducing/preventing adolescent ED should: (i) include parent-focused interventions which consider role modelling, access and supply, and communicating health-promotion messages; (ii) promote and

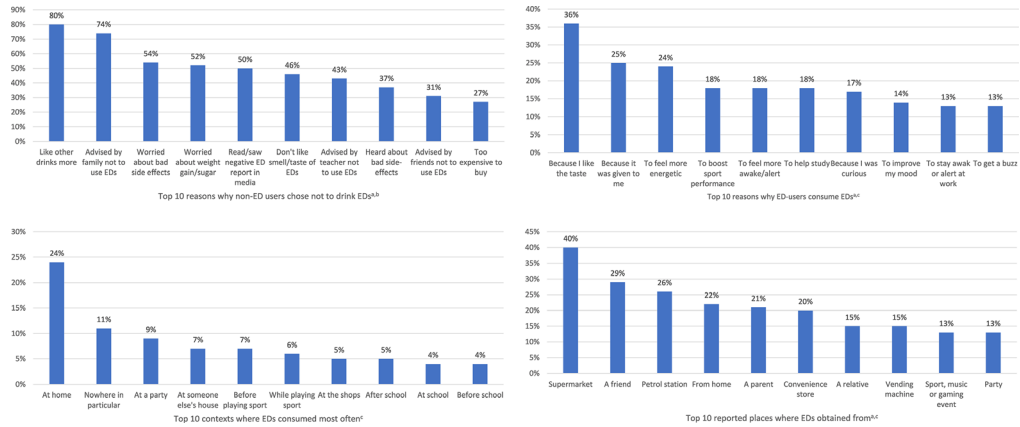

Figure 1 Reasons and context for adolescent energy drink (ED) use and non-use. ED, energy drink. [a]Percentages do not total 100% as respondents could select multiple responses. [b]*n* = 1544 non-ED users (255 missing). [c]*n* = 1686 ED-users (203 missing)

educate young people about alternative (healthy) ways to increase energy levels; (iii) be conducted prior to children turning 10 years, and prior to peak academic stress periods (e.g. exams); and (iv) educate young people about the health dangers associated with consuming EDs when playing sport. Legislative and policy changes including possible licensing of sales (similar to alcohol regulation), may be needed to address adolescents' access to EDs (for a discussion on potential implementation in Australia, see Bromberg & Howard [13] 2016). This regulation would decrease the ability and perhaps desire of minors to purchase and consume EDs. Internationally, several countries have banned EDs outright or placed age-related restrictions on sales [14].

Despite being at risk of selection bias (as a result of active consent) and recall and social desirability bias, the key strengths of the present study include its large sample size (i.e. 3688 adolescents), inclusion of schools with an even representation of school-level socio-economic status (i.e. 50% of participating schools had a high ICSEA score, 50% had a low ICSEA score), inclusion of participants from all secondary school grades (i.e. grades 7 through 12) and inclusion of both metropolitan and regional schools. Furthermore, our sample is broadly representative of the Australian population for this age category. For example, the gender distribution of children 12–17 years in Western Australia is 51% male and 49% female [15], which is consistent with most Australian states and territories and the national distribution [16]. The geographical distribution of our sample (i.e. 77% metropolitan and 23% regional) is also similar to the 74% metropolitan and 26% regional geographical distribution of all children aged 12–17 years within Western Australia [15].

In conclusion, the present study contributes to the limited body of international evidence on adolescent ED use and provides valuable information to help ensure future interventions to reduce/prevent ED consumption and address the underlying reasons and contexts of use.

Transparency Declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with STROBE2 guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

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Conflict of interests, source of funding and authorship.

The authors declare that they have no conflicts of interest.
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All authors contributed to the conceptual design of the research. GT conducted the data analysis and all authors contributed to the interpretation of results. GT and MH drafted the manuscript. All authors critically revised the article for important intellectual content and approved the final version submitted for publication.

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BEVERAGES

Consumption of sugar-sweetened beverages and serum uric acid concentrations: a systematic review and meta-analysis

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Keywords
cross-sectional, fructose, hyperuricemia, meta-analysis, serum uric acid, sugar-sweetened beverages.

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Abstract

Background: Serum uric acid concentration has been linked with metabolic abnormalities. The available evidence on the association of Sugar-Sweetened Beverage (SSB) intake with serum uric acid concentrations is conflicting. The present study aimed to summarise earlier findings on the association of SSB consumption with serum uric acid concentrations in adults.
Methods: Using relevant keywords, we conducted a search in PubMed (https://pubmed.ncbi.nlm.nih.gov), Scopus (https://www.scopus.com) and Google Scholar (https://scholar.google.com) up to September 2017 for all published papers assessing SSB intake and serum uric acid concentrations. SSBs were defined as the dietary intake of Sugar-Sweetened Soft Drinks and Fruit Juice (FJ), or as Sugar-Sweetened Soft Drinks, Diet Soft Drinks and Orange Juice or as Soda and FJ.
Results: After excluding non-relevant papers, five studies, with six effect sizes, remained in our systematic review. All studies included in the current systematic review were of cross-sectional design that were published between 2007 and 2013. The number of participants ranged from 483 to 14 761 people. Most studies had controlled for age, body mass index, weight and sex. We found that individuals in the highest category of SSB intake had 0.18 mg dL^{-1} greater concentrations of serum uric acid compared to those in the lowest category (summary effect size: 0.18 mg dL^{-1}; 95% confidence interval = 0.11–0.25). No significant between-study heterogeneity was found (I^2 = 0.0%, P = 0.698). In the sensitivity analysis, we found no particular study influence on the summary effect. There was no evidence of publication bias.
Conclusions: We found that SSB consumption was significantly associated with increased serum uric acid concentrations in an adult population.

Introduction

Uric acid or urate is an end-product of purine nucleotides of DNA or RNA. It has been considered as one of endogenous antioxidants [(1)]. Increased levels of uric acid, or hyperuricemia, have doubled worldwide during the last few decades [(2)]. Such a significant rise in serum uric acid levels is parallel to an increased prevalence of gout,

rheumatoid arthritis, obesity, diabetes mellitus, hypertension and cardiovascular disease [2,3].

Serum acid uric concentrations are influenced by several modifiable and non-modifiable factors. Possible underlying lifestyle-related factors include diet and obesity [4,5]. Earlier studies suggested that, along with obesity, purine-rich foods such as meat, sea-foods and alcohol might play a key role in developing hyperuricemia [6]. The consumption of other foods including dairy products and dietary sources of vitamin C was protectively linked with hyperuricemia [1]. Recently, the consumption of Sugar-Sweetened Beverages (SSBs) and fructose-rich drinks has received considerable attention in relation to over-production of uric acid and hyperuricemia [1,5,7]. However, there is no clear dietary recommendation about intakes of fructose and SSBs in hyperuricemic patients. Several studies have examined the link between consumption of fructose and SSBs and serum uric acid levels, although the findings are conflicting. Data from cross-sectional studies revealed that increased intakes of SSBs and fructose were associated with increased serum uric acid levels [2,8]. Other studies found a positive association with SSB consumption but not with fructose intake [5]. In a randomised, controlled cross-over trial, the consumption of high fructose corn syrup, compared to sucrose-sweetened soft drinks, resulted in increased post-prandial concentrations of serum uric acid levels in healthy subjects [10]. Some studies have reported a gender difference in this association. A positive significant association between SSB intake and serum uric acid concentrations was seen in males but not in females [9]. Some other studies have failed to reach a significant association [1,5,6]. Although, SSBs contain low purine levels, they contain large amounts of fructose, which can in turn increase serum uric acid levels [9]. Despite earlier investigations on the relation between intake of SSBs and serum uric acid concentrations, we are aware of no comprehensive systematic review or meta-analysis summarising earlier findings in this regard. To address this, we conducted the current systematic review and meta-analysis to summarise the available data about the association between consumption of SSBs and serum levels of uric acid.

Materials and methods

Search strategy

This systematic review and meta-analysis of observational studies were conducted on studies published up to September 2017. This systematic search was performed in PubMed (https://pubmed.ncbi.nlm.nih.gov), Scopus (https://www.scopus.com) and Google Scholar (https://scholar.google.com) by two independent investigators to identify relevant articles. Grey literature, including conference papers and theses, was not included. The relevant keywords (text words and MeSH and non-MeSH terms) that were used in our search were: Pubmed search strategy: ('levulosagrifols' OR 'levulosabraun' OR 'apirlevulosa' OR 'ern brand of fructose' OR 'levulosafleboplast' OR 'levulosadovitulia' OR 'braun brand of fructose' OR 'levulosaibys' OR 'fructose' OR 'levulose' OR 'plastapyrlevulosamein' OR 'grifols brand of fructose' OR 'levulosaapir' OR 'levulosamein' OR 'fleboplastlevulosa' OR 'baxter brand of fructose' OR 'levulosa' OR 'levulosadobieffemedit' OR 'levulosaife' OR 'institutofarmacologico brand of fructose' OR 'levulosadobraun' OR 'freseniuskabi brand of fructose' OR 'levulosabaxter' OR 'bieffe brand of fructose' OR 'sugar-sweetened beverages' OR 'soft drink' OR 'soft drinks' OR 'beverage' OR 'beverages' OR 'carbonated soft drinks' OR 'fruitades' OR 'fruit drinks' OR 'sports drinks' OR 'energy and vitamin water drinks' OR 'sweetened iced tea' OR 'punch' OR 'fruit punch' OR 'cordials' OR 'squashes' OR 'lemonade' OR 'soda' OR 'soda-pop') AND ('gout' OR 'hyperuricemia.mp.' OR 'uric.mp.' OR 'exp uric acid' OR 'urate.mp.'OR 'expurate'). SCOPUS search strategy: (ALL('levulosagrifols') OR ALL ('levulosabraun') OR ALL('apirlevulosa') OR ALL('ern brand of fructose') OR ALL('levulosafleboplast') OR ALL ('levulosadovitulia') OR ALL('braun brand of fructose') OR ALL('levulosaibys') OR ALL('fructose') OR ALL ('levulose') OR ALL('plastapyrlevulosamein') OR ALL ('grifols brand of fructose') OR ALL('levulosaapir') OR ALL('levulosamein') OR ALL('fleboplastlevulosa') OR ALL ('baxter brand of fructose') OR ALL('levulosa') OR ALL('levulosadobieffemedit') OR ALL('levulosaife') OR ALL('institutofarmacologico brand of fructose') OR ALL ('levulosadobraun') OR ALL('freseniuskabi brand of fructose') OR ALL('levulosabaxter') OR ALL('bieffe brand of fructose') OR ALL('sugar-sweetened beverages') OR ALL ('soft drink') OR ALL('soft drinks') OR ALL('beverage') OR ALL('beverages') OR ALL('carbonated soft drinks') OR ALL('fruitades') OR ALL('fruit drinks') OR ALL ('sports drinks') OR ALL('energy and vitamin water drinks') OR ALL('sweetened iced tea') OR ALL('punch') OR ALL('fruit punch') OR ALL('cordials') OR ALL ('squashes') OR ALL('lemonade') OR ALL('soda') OR ALL('soda-pop')) AND (ALL('gout') OR ALL ('hyperuricemia.mp.') OR ALL('uric.mp.') OR ALL('exp uric acid') OR ALL('urate.mp.') OR ALL('expurate')). Google Scholar search strategy: ('levulosagrifols' OR 'levulosabraun' OR 'apirlevulosa' OR 'ern brand of fructose' OR 'levulosafleboplast' OR 'levulosadovitulia' OR 'braun brand of fructose' OR 'levulosaibys' OR 'fructose' OR 'levulose' OR 'plastapyrlevulosamein' OR 'grifols brand of fructose' OR 'levulosaapir' OR 'levulosamein' OR 'fleboplastlevulosa' OR 'baxter brand of fructose' OR 'levulosa' OR 'levulosadobieffemedit' OR 'levulosaife' OR 'institutofarmacologico brand of fructose' OR 'levulosadobraun'

OR 'freseniuskabi brand of fructose' OR 'levulosabaxter' OR 'bieffe brand of fructose' OR 'sugar-sweetened beverages' OR 'soft drink' OR 'soft drinks' OR 'beverage' OR 'beverages' OR 'carbonated soft drinks' OR 'fruitades' OR 'fruit drinks' OR 'sports drinks' OR 'energy and vitamin water drinks' OR 'sweetened iced tea' OR 'punch' OR 'fruit punch' OR 'cordials' OR 'squashes' OR 'lemonade' OR 'soda' OR 'soda-pop') AND ('gout' OR 'hyperuricemia.mp.' OR 'uric.mp.' OR 'exp uric acid' OR 'urate.mp.'OR 'expurate'). Finally, a manual search was also performed on the references of the relevant studies to avoid missing any publication.

Inclusion criteria

Studies were independently assessed by two reviewers considering PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. Then we assessed relevant studies for their quality before any data extraction. Any disagreements between the reviewers were resolved through discussion. We included published studies if they were prospective cohort, case-control or cross-sectional studies that reported the mean (SD) or mean (SEM) of serum uric acid. We did not apply any time limitations; however, we limited our search to English papers.

Exclusion criteria

All editorials, letters, comments, ecological studies, randomised controlled trials and meta-analyses were excluded from our search. We did not include unpublished data. Duplicate citations were excluded. Following our initial search, 3457 published articles were identified. After removing 202 duplicates, 3255 abstracts were selected for a more detailed review; of these, 3233 papers were excluded after screening for titles and abstracts. From the remaining 22 relevant papers, 12 articles were finally selected for detailed reviewing. The excluded 10 papers had been published in public media or in a non-English language (German or Italian). In addition, out of 12 relevant studies, seven papers were again excluded for specific reasons: two studies were excluded because of the lack of reporting any effect size or confidence intervals (CIs) [11,12]. Studies by López-Molina *et al.* [13] and Murphy *et al.* [14] were excluded because they did not report any data for the relation between SSBs and serum uric acid levels. In addition, a study by Ferraro *et al.* [15] had assessed the relationship between soda consumption and kidney stones; however, any relevant data about the outcomes were not reported. Studies by Bobridge *et al.* [16] and Lin *et al.* [17] were excluded because their study populations comprised adolescents. After these exclusions, five papers remained for the current systematic review and meta-analysis. Figure 1 illustrates the study selection process for the systematic review and meta-analysis.

Data extraction

Data extraction was carried out independently and cross-checked by two reviewers (SE and PS). Disagreements between the two reviewers were considered by the principal investigator (AE). Quantitative data regarding effect size measures such as the mean difference (MD) and 95% CI or geometric mean and mean (SD) or median (interquartile range) were extracted. The extracted data comprised all details about the study question. Extracted data from cross-sectional studies included the first author's last name, publication year, the country in which the study was conducted, age range or mean age of participants at baseline, sex, sample size, type of exposure, assessment methods of exposure, main outcomes, assessment methods of outcomes, comparison of exposures, potential covariates adjusted for in the analyses and quality score of Newcastle-Ottawa Scale. We also contacted corresponding authors for key information when data were ambiguous or missing from the published study.

The serum uric acid levels of all studies were converted to mg dL^{-1}. In the study by Zgaga *et al.* [5], serum uric acid levels were reported as mmol dL^{-1}. After converting to mg dL^{-1}, we found that the values were markedly different from the other published studies. Therefore, we contacted the authors to resolve this problem; however, we did not receive any response from them. We assumed that there was a typographical error in their publication and the values reported in their paper were probably in units of μmol dL^{-1}, rather than mmol dL^{-1}. Therefore, all of the values in their publication were considered as μmol dL^{-1} and then converted to mg dL^{-1}.

Quality assessment

Two reviewers independently re-checked each selected article to minimise bias. All selected articles were judged for their quality based on the Newcastle-Ottawa Scale adapted for cross-sectional studies. The quality scores by Newcastle-Ottawa Scale were derived by considering three aspects of each study, including selection, comparability and outcome in eight questions. Each study could be awarded a maximum score of 1 for each question in the selection and outcome part. However, a maximum score of 2 could be given for the comparability question. Scores of more than 6 were considered as good quality. The maximum score was 10.

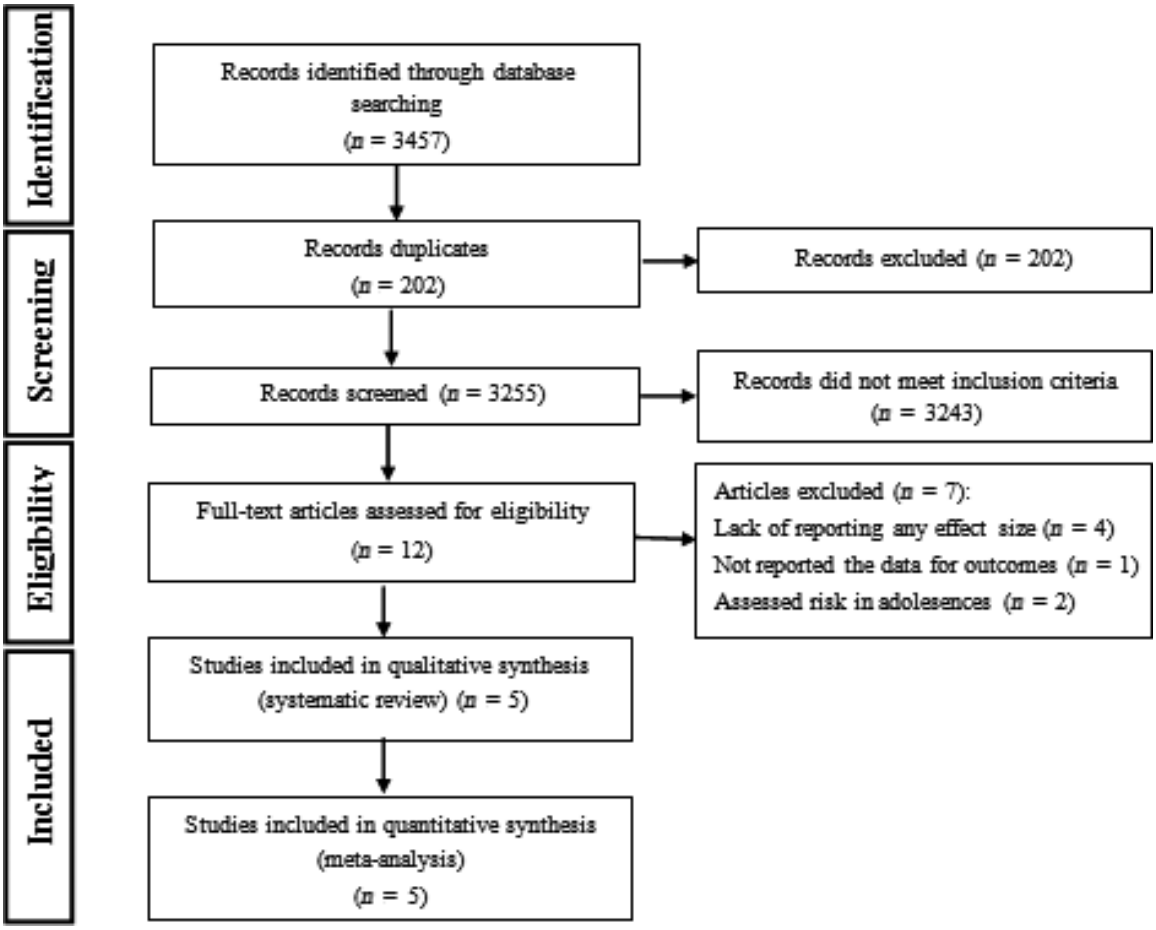

Figure 1 Flowchart of study selection process.

Statistical analysis

MD and SDs of serum uric acid levels were used for the meta-analysis. Included studies had provided serum uric acid concentrations for the comparison of the highest versus the lowest category of sugar-sweetened beverages intake. If a certain study had not provided the MD of serum uric acid concentrations, we computed it by considering the levels for the highest and the lowest intakes of SSBs. Some studies had reported geometric means and the mean (SD) or mean (SE) [1,5,9]. We used SEs and SDs of each study for calculating SE or SD for MD. Summary estimates and their SDs were derived by the method of DerSimonian and Laird. We conducted random-effects model for performing the meta-analysis and calculated both Cochran's *Q*-statistics and I^2 as indicators of heterogeneity. Publication bias was examined by visual inspection on Begg's funnel plots. We performed the formal statistical assessment of funnel plot asymmetry using Egger's regression asymmetry test. Sensitivity analysis was conducted in which each cross-sectional study was excluded to evaluate the influence of that study on the overall results. We used STATA, version 11.2 (StataCorp LP) for all of the statistical analyses. $P < 0.05$ was considered statistically significant.

Results

Findings from the systematic review

Out of 3255 papers, we found five cross-sectional studies that reported the association of SSB intake with concentrations of serum uric acid in adults. Table 1 illustrates the main characteristics of studies that examined the association of SSB intake with serum uric acid levels. These studies were published between 2007 and 2013. Three studies were conducted in USA [8,9,18], one in Scotland [5] and one in Singapore [6]. The age range of participants was ≥12 years. All studies were performed on both genders. Number of participants ranged from 483 to 14 761 people. SSBs were defined as dietary intake of Sugar-Sweetened Soft Drinks (SSD) and Fruit Juice (FJ) [9], or as Sugar-Sweetened Soft Drinks (SSD), Diet Soft Drink

(DSD) and Orange Juice [18] or as Soda and FJ [6]. Two studies had considered total SSB intake without mentioning its components [5,8]. Three studies had used a food frequency questionnaire for dietary assessment [5,6,18]. The studies by Nguyen *et al.* [8] and Gao *et al.* [9] had used simple 24-h recalls for dietary assessment. The outcome of interest was serum uric acid in all these publications, except for the study by Zgaga *et al.* [5], which had considered urate concentrations as the outcome. All studies had examined serum uric acid concentrations by laboratory enzymatic assay. Two studies had reported the mean (SD) or mean (SE) of serum uric acid concentrations across categories of SSB intake [5,9] and three studies had reported mean difference of serum uric acid concentrations comparing extreme categories [6,8,18].

Most studies had controlled for age, body mass index, weight and sex (when relevant). Moreover, further controlling for total energy intake, smoking, alcohol intake, diuretic use and purin-rich foods was also performed in some studies. Additional adjustment for physical activity, vitamin C, fruit and vegetable intake, fasting blood sugar, fluid intake, glomerular filtration rate, caffeine and tea intake was also considered in some studies. With regard to the study findings, almost all studies reported a positive association between SSB consumption and serum uric acid levels, except for the study by Teng *et al.* [6] that had reported a significant negative association.

Findings from the meta-analysis

Combining six effect sizes from five cross-sectional studies [5,6,8,9,18], we found that SSB consumption was significantly associated with increased levels of serum uric acid concentrations; such that individuals in the highest category of SSB intake had 0.18 mg dL^{-1} greater concentrations of serum uric acid compared to those in the lowest category (summary effect size: 0.18 mg dL^{-1}; 95% CI = 0.11–0.25) (Fig. 2). This result was not changed after excluding the study by Zgaga *et al.* [5] from the meta-analysis (summary effect size: 0.18 mg dL^{-1}; 95% CI = 0.11–0.25). There was no significant between-study heterogeneity (I^2 = 0.0%, P = 0.698). No particular study had a significant influence on the summary effect. There was no proof of significant publication bias. Sub-group analysis based on US countries and non-US countries was peformed. There was a significant association between highest intake of SSBs and greater levels of serum uric acid among the US population (summary effect size: 0.18 mg dL^{-1}; 95% CI = 0.11–0.26) (Fig. 3). No significant association was seen among non-USA people in this regard (summary effect size: 0.10 mg dL^{-1}; 95% CI = −0.16 to 0.36) (Fig. 3).

Discussion

Based on six effect sizes of five cross-sectional studies [5,6,8,9,18], we found a significant positive association between SSB consumption and serum uric acid levels. This finding supports the hypothesis that a high consumption of SSBs might be associated with increased serum uric acid concentrations. To the best of our knowledge, this is the first systematic review and meta-analysis summarising the association of SSB intake and elevated serum levels of uric acid in adults. To interpret our findings, it must be kept in mind that some studies reporting the effect sizes separately for different SSBs in the same population were combined before considering them in the final analysis.

Despite being an endogenous antioxidant, recent studies have indicated that high serum uric acid concentrations may contribute to the incidence of metabolic syndrome, diabetes, hypertension and cardiovascular disease [1]. Concentrations of serum uric acid may vary significantly among populations. Among dietary factors, fructose as a component of SSBs might influence the production of uric acid [6]. We found that an increased consumption of SSBs was associated with high levels of serum uric acid concentrations. Our finding was in line with several reports from observational studies that showed a significant positive association between SSB intake and increasing serum levels of uric acid [5,8,9,18]. By contrast, some studies failed to find a significant positive association. Teng *et al.* [6] found a significant inverse association between SSB consumption and serum uric acid levels. However, the sample size in their study was small compared to other studies and they had considered just soda and fruit juice in their study protocol.

The possible mechanisms through which SSB consumption might influence serum uric acid concentrations are not clear. However, several ideas have been proposed in this regard. SSBs contain high levels of fructose, for which consumption can produce uric acid via increasing ATP degradation to AMP, a substrate for uric acid production [19-22]. Fructose phosphorylation during hepatic metabolism uses ATP and leading to phosphate depletion, which limits the regeneration of ATP from ADP. In turn, ADP converts to AMP and consequently increases the uric acid formation [23]. In addition, oral administration of high doses of fructose induces insulin resistance, impaired glucose tolerance and hyperuricemia [24,25]. Despite these mechanisms, some studies indicated no significant association between dietary fructose intake and serum uric acid levels [1,5]. However, these studies were mostly of cross-sectional design [1,5] and the findings from prospective

Table 1 Main characteristics of cross-sectional studies examined the association of sugar-sweetened beverage intake and serum acid uric levels (mg dL^{-1})

First Author (year)	Country	Range/ mean age	Sex	Sample size	Exposure	Exposure assessment	Outcome	Outcome assessment	Comparison	MD (95% CI)	Mean (SD) or mean (SE)
SSB intake											
Gao *et al*. [9] 2007	USA	>18	M/F	4073	SSB*	Single 24 hour recall	serum acid uric	Beckman Synchron LX20 (oxidation by uricase)	Q4 versus Q1	NR	SSD: 5.66 (0.06) versus 5.31 (0.03)¶ FJ: 5.43 (0.06) versus 5.41 (0.05)¶
Choi *et al*. [18] 2008	USA	>20	M/F	14761	SSB†	FFQ	Serum uric acid	(oxidation by uricase)	SSD and DSD ≥4 versus 0, orange J ≥1 versus 0 (serving/day)	SSD: 0.42 (0.11–0.73) DSD: −0.12 (−0.43–0.19) Orange J: 0.17 (0.01 − 0.34)	NR
Nguyen *et al*. [8] 2009	USA	12-18	M/F	4867	SSB‡	24hour recall	serum acid uric	Colorimetric method (Oxidation by uricase)	SSB ≥36 versus 0 (oz/day)	SSB: 0.18 (0.02–0.33)	NR
Zgaga *et al*. [5] 2012	Scotland	62	M	2076 M:1163	SSB	FFQ	Urate concentration	Laboratory evaluation (NR)	≥2 serving/day versus 0	NR	5.32 (1244.18) versus 5.04 (1138.44)
Zgaga *et al*. [5] 2012	Scotland	62	F	2076 F:913	SSB	FFQ	Urate concentration	Laboratory evaluation (NR)	≥2 serving/day versus 0	NR	4.43 (1396.32) versus 4.33 (1151.88)
Teng *et al*. [6] 2013	Singapore	45-74	M/F	483	SSB§	FFQ	Serum uric acid	Direct enzymatic assay	≥6 times/day versus 0 (Daily drinkers versus nondrinkers)	Soda: 4.99 (4.39–5.67) versus 5.24 (5.06–5.43) FJ: 5.14 (4.59–5.76) versus 5.25 (5.06–5.44)	NR

DB, Diet Beverage; DSD, Diet Soft Drink; F, female; FFQ, food frequency questionnaire; FJ, fruit juice; M, male; NR, not reported; SSB, Sugar-Sweetened Beverage; SSD, Sugar-Sweetened Drink and Sugar Soft Drink.

*SSB including: SSD, FJ.

†SSB including: SSD, DSD, Orange J.

‡SSB including: SSB, DB, FJ.

§SSB including: Soda, FJ.

¶Values are the mean (SE).

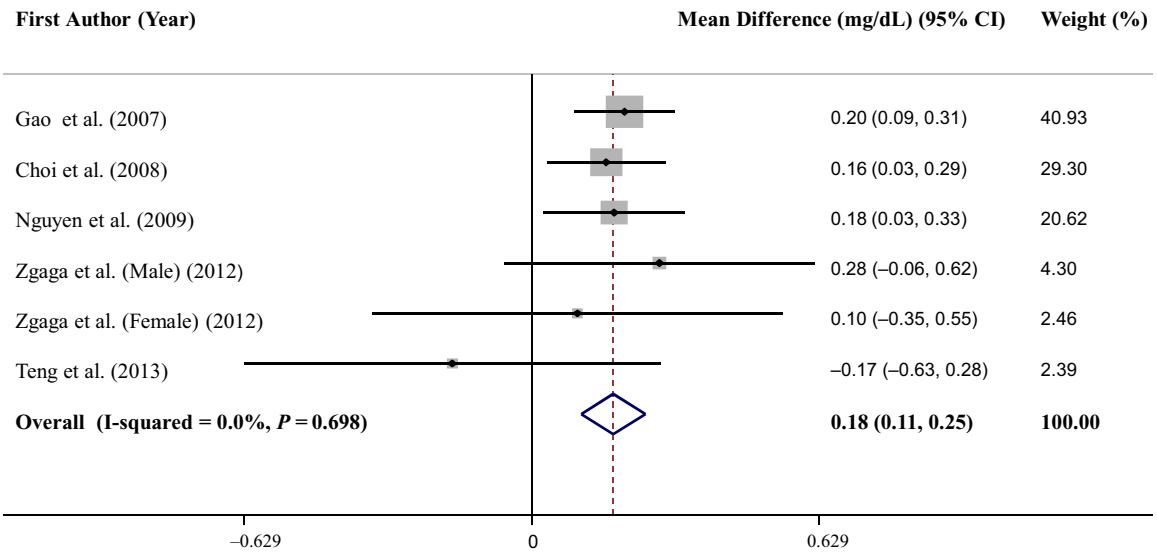

Figure 2 Mean differences for serum uric acid in highest versus lowest category of sugar-sweetened beverage intake.

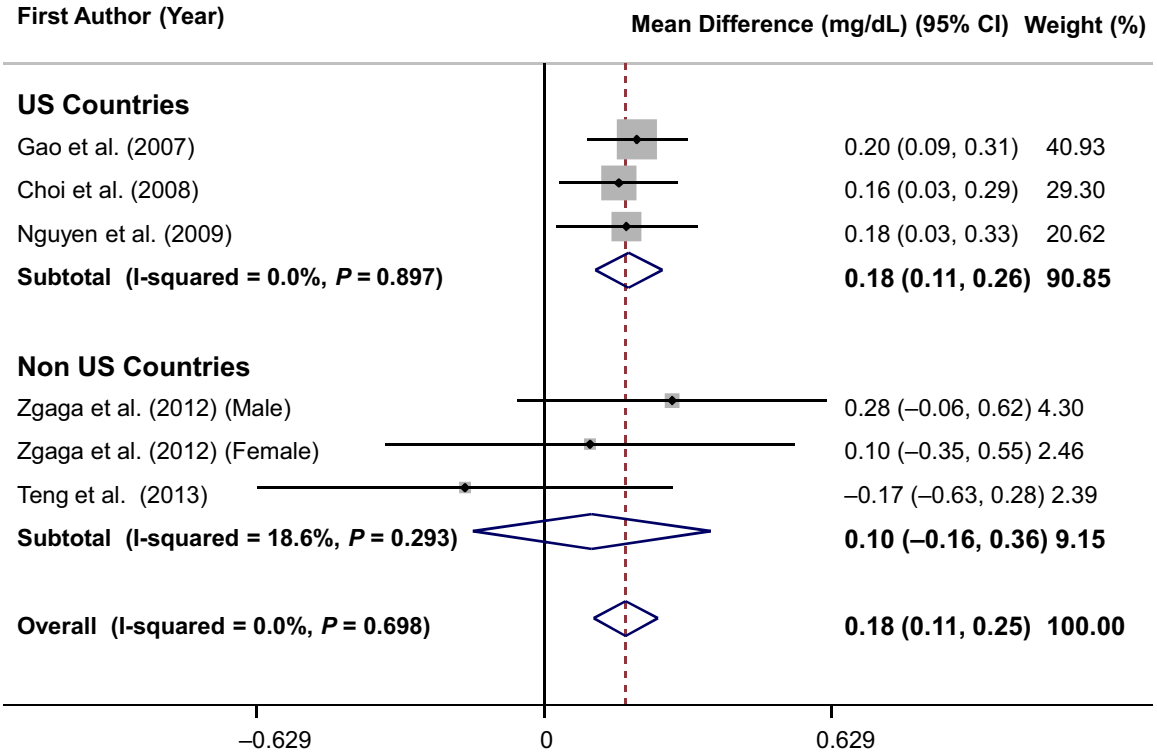

Figure 3 Mean differences for serum uric acid in highest versus lowest category of sugar-sweetened beverage intake in US countries and non-US countries.

cohort studies have reported a positive association between fructose intake and increased risk of gout [19,20]. In addition, it is possible that other ingredients of SSBs, including additives and added colours, might explain the association with increased concentrations of serum uric acid.

Despite being the first meta-analysis on SSB intake and serum uric acid concentrations, some limitations need to be considered. There is a gender difference in serum uric acid levels as a result of the influence of oestrogen in increasing the clearance of uric acid [26,27]. Therefore, it appears that sex-stratified analysis is required to reach a

definite conclusion on the association of SSB intake and serum uric acid levels. However, as a result of the limited number of publications, we did not perform sub-group analysis by gender. Despite the study by Nguyen *et al.* [8] being performed on 12–18 year-olds, we included it in our systematic review and meta-analysis because it considered individuals who were 18 years old and it was not possible to specify the findings only for adolescents. In addition, it must be kept in mind that the current meta-analysis was performed on cross-sectional studies as a result of the lack of sufficient number of prospective cohort studies in this regard. Therefore, the results should be interpreted cautiously.

Conclusions

In summary, the findings of the present meta-analysis indicated that SSB consumption was significantly associated with increased serum acid uric concentrations. Further studies are required to clarify the association between SSB consumption and serum uric acid concentrations.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with PRISMA guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained. Present systematic review was registered in PROSPERO systematic review registry (registry code number: CRD42020168518).

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Conflict of interest, source of funding and authorship

The authors declare that they have no conflicts of interest.
The project was supported by the Tehran University of Medical Sciences.
SE and PS contributed to the study conception, design, search, statistical analyses, data interpretation and manuscript drafting. BL contributed to design and data interpretation. AE contributed to study conception, design, statistical analyses, data interpretation and manuscript drafting. AE supervised the study. All authors approved the final version of the manuscript submitted for publication.

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MICRONUTRIENTS

Association of iodine-related knowledge, attitudes and behaviours with urinary iodine excretion in pregnant women with mild iodine deficiency

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Abstract

Background: Subsequent to the implementation of the universal salt iodisation policy, China has all but eliminated the iodine deficiency disorders. However, pregnant women are still experiencing mild iodine deficiency. The present study explored factors that could relate to mild iodine deficiency in pregnant women.
Methods: In total, 2400 pregnant women were enrolled using a multistage, stratified, random sampling method in Shanghai. Data were collected via a standardised questionnaire. The urine samples and household cooking salt samples were collected for the detection of urinary iodine and salt iodine concentrations.
Results: The median urinary iodine concentration (MUIC) was 148.0 μg L^{-1} for all participants, and 155.0 μg L^{-1}, 151.0 μg L^{-1} and 139.6 μg L^{-1} in the first, second and third trimesters. The MUIC in the third trimester was significantly lower than that of the first trimester ($P < 0.05$). The usage rates of iodised salt and qualified-iodised salt were 71.5% and 59.4%, respectively. Iodine-related knowledge score composition ratio was significantly different between the high and low UIC groups ($P < 0.05$). Participants' MUIC increased significantly with the increases in iodine-related knowledge score ($P < 0.001$). The third trimester was a significant risk factor for high UIC, whereas high iodine-related knowledge score, actively learning dietary knowledge and having a habit of consuming iodine-rich food were significant protective factors for high UIC ($P < 0.05$).
Conclusions: Iodine level is adequate among pregnant women in Shanghai during the first and the second trimesters, although it is is insufficient in the third trimester. Good iodine-related knowledge, attitudes and behaviours are important for pregnant women with respect to maintaining adequate urinary iodine.

Introduction

Iodine is a traceable element that is essential for thyroid hormone synthesis, as required for infants' physical growth and mental development [1,2]. Iodine deficiency can lead to a series of adverse results, including endemic goitre and poor physical growth, collectively known as iodine deficiency disorders (IDD). Severe iodine deficiency in pregnancy is well known to result in adverse childhood outcomes, such as cretinism and mental

retardation [3]. In recent decades, IDD have become the most prevalent micronutrient-related diseases worldwide; approximately 30% (1.9 billion) of the global population (especially children and pregnant women) suffer from different levels of iodine deficiency [4]. Iodised salt has been introduced in many countries to eliminate IDD, including Switzerland, Sweden, Mongolia, China, and so on. Thus, areas with severe deficiency have become uncommon and the concern has shifted toward mild to moderate iodine deficiency [5–6].

The national monitoring of China showed that, after the implementation of the compulsive universal salt iodisation (USI) policy, population iodine levels were adequate except in pregnant women [7]. Many studies suggest that even mild iodine deficiency during pregnancy may negatively affect the verbal intelligence quotient and educational level of offspring [8–9]. Not only in China, but also in many other countries, pregnant women have suffered from mild iodine deficiency. The median urinary iodine concentration (MUIC) of pregnant women was 129 μg L^{-1} in the US National Health and Nutrition Examination Survey 2005–2010, which indicated an insufficient iodine status [10]. In 2019, the MUICs of pregnant women in Britain during their three trimesters were only 94, 117 and 90 μg L^{-1}, respectively [11].

Although Shanghai is a developed coastal city, it is also an iodine deficiency area with low iodine concentrations in the drinking water (12.8 μg L^{-1}). According to the latest Chinese standard implemented in 2020, the main source of iodine for Shanghai residents was iodised salt, followed by iodine supplements and iodine-rich foods [12,13]. Urinary iodine excretion in spot urine samples is the easiest method for assessing iodine deficiency and a low-median value suggests that a population is at higher risk of developing thyroid disorders [13]. Shanghai has followed the national compulsive USI policy from 1996 onwards and the city began monitoring the iodine status of children 8–10-year-old in 1995 and pregnant women in 2009. According to the monitoring data, the iodine status of Shanghai's 8–10-year-old children was adequate after 2005, although the MUIC of pregnant women has indicated mild iodine deficiency, with a level of only 135.9 μg L^{-1} in 2009, 139.8 μg L^{-1} in 2012 and 126.5 μg L^{-1} in 2015 [13–15]. Furthermore, monitoring in Shanghai has revealed that more and more families choose non-iodised salt as their cooking salt, and the rate of household using iodised salt has dropped from 94.6% in 1999 to 76.5% in 2017 [15]. Hence, the iodine level in pregnant women has become a concern.

From 2017 onward, China's salt industry has undergone reforms, allowing businesses to be conducted across regions, which has increased difficulties of the industry management and supervision. Merchandising of non-iodised salt has shifted from only through government-qualified channels to other more accessible channels (e.g. the Internet). On the other hand, some residents lack iodine knowledge, not knowing the harm caused by iodine deficiency and whether their iodine intake is insufficient. Hence, a growing number of households are more likely to buy non-iodised salt which has then reduced the consumption rate of iodised salt, for lack of iodine-related knowledge, attitudes and behaviours. Given these developments, the present study aimed to systematically assess the iodine status of pregnant women in Shanghai during 2019 and explore iodine-related knowledge, attitudes and behaviours with the potential associated mild iodine deficiency in pregnant women.

Materials and methods

Population and study design

The data were retrieved from the results of Shanghai's annual IDD surveillance, from March to June 2019, comprising a cross-sectional study. The target population was pregnant women with at least twelve continuous months of residency in Shanghai. A multistage, stratified random sampling method was conducted in 16 districts of Shanghai. According to the formula for calculating stratified random sampling sample size, at least 1537 pregnant women were needed for analysis. Each district was divided into five sections, and one street was randomly selected from each section, and then 30 pregnant women were selected from each selected street. Women with different gestational weeks were evenly distributed.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Shanghai Municipal Centre for Disease Control and Prevention (CDC). Written informed consent was obtained from all participants

Questionnaire survey

All participants were required to complete a standardised questionnaire, which included information on demographics, iodine-related knowledge, attitudes and behaviors through face-to-face interviews with trained interviewers (see Supporting information, Table S1). Participants' iodine-related knowledge was measured using a questionnaire with 8 items and a total possible score of 10 points. The items included 'Is iodine an essential macronutrient or micronutrient (one point)', 'What is the most safe, cost-effective and sustainable way to eliminate IDD (one point)', 'Do you think excessive iodine intake is harmful to human health (one point)', 'Does Shanghai belong to iodine-deficient area (one point)',

'Do pregnant women in Shanghai need iodine supplementation in the preparation stage of pregnancy (one point)', 'Do pregnant women in Shanghai need iodine supplementation during pregnancy (one point)', 'Which hormone's synthesis does iodine has participate in (two points)' and 'Which food contains the most iodine content (two points)'. Each item had three to five options but only one correct answer. Participants obtained one or two points for a correct answer and 0 points for a wrong answer. Iodine-related knowledge score was given as each participant's total score on the questionnaire. The reliability coefficient of the questionnaire was 0.87, which was considered acceptable. Using the answering condition of 'Are you actively learning dietary knowledge?' and 'Do you have a habit of consuming iodine-rich food including salt iodisation?' to evaluate participants' iodine-related attitudes and behaviors. All data were primarily reviewed by the local district CDC project team, whereas the Shanghai CDC project team reviewed at least 5% of the data.

Data collection and analyses of household cooking salt and urine samples

More than 50g of household cooking salt and 5 mL of random urine from each subject were collected. Salt samples were collected by investigators, and the urine samples were collected by the subjects themselves and then placed into the iodine-free cryopreserved tube. All urine samples were first temporarily stored in a refrigerator at 4°C, then stored in a freezer at −20 °C within 12 h and, finally, transported to the testing unit within 2 weeks according to the requirements of national inspection standards [16]. All cooking salt samples were stored at room temperature and transported to the laboratory within 1 week.

The household cooking salt iodine concentration (SIC) and urinary iodine concentration (UIC) were measured through titration and acid digestion [16,17], respectively, at the central laboratory of Shanghai's Municipal CDC and 16 district CDCs in Shanghai. The internal quality control of the samples for the analyses of the SIC and UIC were provided by China's National Iodine Deficiency Disorders Reference Laboratory of the CDC. All results of the internal quality control samples were qualified.

Definitions and classifications of relevant indicators

Cooking SIC was classified into two types based on cooking SIC standards in Shanghai: non-iodised salt (SIC <5.0 mg kg^{-1}) and iodised salt (SIC ≥ 5.0 mg kg^{-1}) which include low-iodised salt (5.0 mg $kg^{-1} \leq$ SIC <21.0 mg kg^{-1}) and qualified-iodised salt (SIC ≥ 21.0 mg kg^{-1}). The usage rate of iodised salt was equal to the percentage of salt with an iodine level ≥ 5.0 mg kg^{-1} in all samples. The usage rate of qualified-iodised salt was equal to the percentage of salt with an iodine content ≥ 21.0 mg kg^{-1} in all samples.

The 'high knowledge score' group consisted of participants who scored 8–10 points on the iodine-related knowledge questionnaire. The 'general knowledge score' group were those who scored 6–7 points on the iodine-related knowledge questionnaire. The 'poor knowledge score' group were those who scored ≤5 points on the iodine-related knowledge questionnaire.

'Former smokers' were the participants who smoked cigarettes in the past, excluding those who took a few tentative puffs. 'Former drinkers' were the participants who usually drank alcoholic beverages during non-gestational periods, excluding those who sipped some wine.

The iodine status of the pregnant women was determined by using the recommended criteria of the World Health Organization (WHO)/United Nations Children's Fund (UNICEF)/International Council for the Control of Iodine Deficiency Disorders (ICCIDD). Insufficient iodine intake was defined as MUIC <150 μg L^{-1}, whereas the adequate iodine intake as MUIC 150–249 μg L^{-1} and iodine intake above the requirement as MUIC 250–499 μg L^{-1} [18].

Under the consideration of the high within-person variability of a single spot urine, the WHO programme guide has limited the use and interpretation based on single spot urine per participant to the population median of a sufficiently large group (generally >30) [12]. In our study, the sampling error (95% confidence interval of the MUIC) was considered and calculated using bootstrapping. Pregnant women are divided into 48 units according to the district and trimester. When the upper cut-off level of the MUIC 95% confidence interval in one unit was higher than 150 μg L^{-1}, the iodine status of all pregnant women was considered optimal in this unit. These pregnant women are categorised as high UIC, whereas the rest were considered low UIC.

The definition of having a habit of consuming iodine-rich food was those who consumed at least 50 g of iodine-rich food per month according to the China national monitoring programme. The iodine-rich foods included kelp, laver, seaweed and shrimp, which are based on the food composition tables published by the China Nutrition Society [19].

Statistical analysis

Statistical analyses were conducted with EXCEL 2010 (Microsoft Corp., Redmond, WA, USA) and SPSS, version 21.0 (IBM Corp., Armonk, NY, USA). The frequency count data were expressed as number and percentage

(%), normally distributed data were expressed as the mean (SD) and non-parametric data were expressed as the median (25th percentile, 75th percentile). One-way analysis of variance (ANOVA) was used to compare multiple groups. In pairwise comparisons, homogeneity of variance was tested using the least significant difference test, and heterogeneity of variance was assessed using Tamhane's T2 test. The Kruskal–Wallis one-way ANOVA was used to compare the non-parametric data of multiple groups. Participants were divided into two groups: the high UIC and the low UIC. The UIC group was used as the dependent variable, and variables that are significant in the univariate analysis are used as the independent variables in the binary logistic regression. The variables with multiple classifications were treated as dummy variables. The stepwise method was used in the mode. The criterion for inclusion in the multivariate regression model was 0.05, and the criterion for exclusion was 0.10. $P < 0.05$ was considered statistically significant.

Results

Characteristics of the participants by trimester

In total, 2400 eligible participants were included in the present study. The MUIC was 148.0 μg L^{-1} for pooled participants, and 155.0 μg L^{-1}, 151.0 μg L^{-1} and 139.6 μg L^{-1} for women in the first, second, and third trimesters, respectively. A significant difference was found in the MUICs at different gestational weeks ($P = 0.027$). The pairwise comparisons identified a significant difference only between the first and third trimesters, with a higher MUIC during the first trimester; the adjusted *P*-value was 0.022. Out of a total of 1715 households that used iodised salt as cooking salt, 1426 used qualified cooking salt. The usage rates of the iodised salt and qualified-iodised salt were 71.5% and 59.4%, respectively. The mean iodine content in the iodised salt was 23.9 mg kg^{-1}.

During the research, criteria including age, educational status, occupational status, family income during the past year, thyroid disease history, drinking and smoking habits, iodine-related knowledge, attitudes and behaviors were analysed (Table 1). Comparisons of participants' characteristics by pregnancy trimester revealed a significant difference in educational status among the women in the different trimesters ($P = 0.005$). No significant differences in other characteristics were found.

Iodine-related knowledge score, usage rates of iodised salt, actively learning dietary knowledge and having a habit of consuming iodine-rich food were significantly between the two UIC groups ($P < 0.05$).

The distributions of urinary iodine concentrations and median urinary iodine concentrations among women with different knowledge scores

The percentages of UICs ranged between 150 and 250 μg L^{-1} were 26.1%, 25.8% and 23.1% for the participants in the first, second and third trimesters, respectively, whereas it was 25.0% for the pooled results (Fig. 1).

The MUICs of the participants, which varied by their iodine-related knowledge scores, were 133.5 μg L^{-1} in the 'poor knowledge score' group, 146.0 μg L^{-1} in the 'general knowledge score' group and 164.0 μg L^{-1} in the group with 'high knowledge score' (Fig. 2). A significant difference was found among the MUICs of the three knowledge score groups in the pooled participants ($P < 0.001$). The adjusted *P*-values for the pairwise comparisons were 0.046 between the 'poor knowledge score' and 'general knowledge score' groups, <0.001 between the 'poor knowledge score' and 'high knowledge score' groups and 0.035 between the 'general knowledge score' and 'high knowledge score' groups. The result showed that the MUIC is increased with increases in knowledge scores.

Because trimester was found to be a predictor of UIC, we controlled the impact of trimester on UIC by comparing the groups with different scores in the same trimester. The differences in the MUIC between the groups with different scores in the same trimester were compared using the Kruskal–Wallis test, which found significant differences between the groups with different knowledge scores in the first ($P < 0.001$) and third ($P = 0.012$) trimesters. The pairwise comparisons of the groups with different scores in the first and third trimesters showed a significant difference only between the 'poor knowledge score' and the 'high knowledge score' groups ($P < 0.001$ and $P = 0.009$).

Analysis of the factors associated with urinary iodine concentration

The result of binary logistic regression has shown four variables in the model: (i) iodine-related knowledge; (ii) having a habit of consuming iodine-rich food; (iii) actively learn dietary knowledge; and (iv) the trimester (Table 2). Compared to the first trimester, the third trimester was found as a risk factor for high UIC ($P = 0.016$). Iodine-related knowledge score high versus low ($P < 0.001$), having a habit of consuming iodine-rich food versus not having the habit on it ($P = 0.046$) and actively learn dietary knowledge versus not actively learn ($P = 0.002$) served as protective factors for high UIC.

Table 1 Characteristics of the study participants stratified by gestational weeks and urinary iodine concentration

	First trimester			Second trimester			Third trimester			Pooled			Compared by trimester		Pooled data compared by UICs	
	Low UIC	High UIC	Total	Low UIC	High UIC	Total	Low UIC	High UIC	Total	Low UIC	High UIC	Total	F	*P*	t/F	*P*
N	344	468	802	348	453	801	368	429	797	1050	1350	2400	/	/	/	/
UIC (μg L^{-1}) (Median [P25, P75])	–	–	155.0 (91.0,257.3)	–	–	151.0 (87.1,242.0)	–	–	139.6 (80.0,234.0)	–	–	148.0 (86.5,244.6)	7.189	0.027	–	–
SIC in iodised salt (mg kg^{-1}), mean (SD)	24.3 (4.5)	24.1 (5.3)	24.2 (5.0	23.8 (4.9)	23.6 (4.8)	23.7 (4.9	23.9 (4.4)	23.9 (4.1)	24.0 (4.3	24.0 (4.6)	23.9 (4.8)	23.9 (4.7	1.294	0.274	0.105	0.746
Usage rate of iodised salt (%)	68.2	74.2	71.3	71.1	74.4	72.8	68.5	72.3	70.3	69.3	73.7	71.5	1.255	0.534	5.679	0.017
Usage rate of approved iodised salt (%)	57.0	61.7	59.5	59.0	60.0	59.6	57.6	61.1	59.2	57.9	61.0	59.4	0.020	0.990	2.375	0.123
Age ≥35 years (%)	14.3	12.2	13.2	13.6	11.9	12.7	9.3	10.9	10.0	12.3	11.7	12.0	4.440	0.109	0.214	0.644
Having a habit of consuming iodine-rich food (%)	56.7	43.3	39.8	35.7	37.0	36.3	33.1	38.0	35.4	34.8	39.5	37.2	3.664	0.160	5.631	0.018
Actively learn dietary knowledge (%)	78.6	85.9	82.4	78.9	82.1	80.5	80.2	86.4	83.1	79.3	84.8	82.0	1.886	0.390	12.313	< 0.001
Former smoker (%)	2.6	2.6	2.6	2.0	2.5	2.2	1.4	1.4	1.4	2.0	2.2	2.1	3.165	0.205	0.126	0.723
Former drinker (%)	6.0	8.4	7.2	9.0	8.4	8.7	7.0	5.7	6.4	7.3	7.3	7.5	3.272	0.195	0.038	0.846
Occupational status (%)																
Physical	23.4	29.4	26.6	26.9	24.3	25.6	27.5	32.3	29.7	26.0	28.6	27.3	3.783	0.151	2.019	0.155
Mental	76.6	70.6	73.4	73.1	75.7	74.4	72.5	67.7	70.3	74.0	71.4	72.7				
Educational status (%)																
≤9 years	9.6	11.7	10.7	17.3	15.9	16.7	16.3	12.2	14.6	14.5	13.3	14.0	14.950	0.005	1.703	0.427
Senior high school and college	37.0	39.7	38.4	37.7	40.7	39.4	35.9	37.2	36.7	37.0	39.6	38.2				
Bachelor's degree and above	52.6	48.6	50.5	44.7	42.9	43.9	47.1	50.3	48.7	48.5	47.1	47.8				
Family income in the past year, YUAN (%)																
<100 000	12.8	14.8	13.8	17.3	10.9	14.3	12.6	12.0	12.3	14.2	12.6	13.6	7.588	0.108	2.659	0.265
100 000/200 000	32.2	35.2	33.9	35.7	41.3	38.6	39.4	40.0	39.6	35.8	38.7	37.4				
≥200 000	55.0	49.3	52.3	46.5	47.8	47.1	48.0	48.0	48.1	49.6	48.3	49.0				
Iodine-related knowledge (%)																
Poor	36.2	25.8	30.8	33.7	30.5	32.1	31.9	23.6	28.1	33.9	26.7	30.3	3.461	0.484	18.961	< 0.001
General	32.3	32.8	32.5	32.2	32.5	32.3	33.8	32.1	33.0	32.8	32.5	32.6				
High	31.5	41.4	36.7	34.2	37.0	35.6	34.3	44.3	38.9	33.4	40.8	37.1				
Thyroid disease history (%)																

Table 1 Continued

		First trimester			Second trimester			Third trimester			Pooled			Compared by trimester		Pooled data compared by UICs	
		Low UIC	High UIC	Total	Low UIC	High UIC	Total	Low UIC	High UIC	Total	Low UIC	High UIC	Total	F	*P*	t/F	*P*
No history	thyroid disease	87.8	89.5	88.7	88.9	87.1	88.0	87.9	90.5	89.1	88.2	89.0	88.6	0.457	0.796	0.371	0.542
Had thyroid disease		12.2	10.5	11.3	11.1	12.9	12.0	12.1	9.5	10.9	11.8	11.0	11.4				

P25, 25th percentile; P75, 75th percentile; UIC, urinary iodine concentration; SIC, salt iodine concentration

Discussion

It is particularly important for pregnant women to have an adequate iodine intake, which is not only related to pregnancy outcomes, but also to the mental and physical development of the foetus [20]. Recently, continuous reports have documented iodine deficiency in pregnant women in China [7,21,22]. The Chinese CDC conducted a study on the relationship between thyroid disease and different levels of iodine intake, which indicated low coverage of iodised salt in the coastal cities of China and low iodine concentrations in the environment; the incidence rate of thyroid dysfunction and the UICs demonstrated a U-shaped curve [21]. All of these findings suggest that the iodine status of pregnant women is worthy of attention, especially in regions where both iodine in the drinking water and the usage of iodised salt are low.

Although Shanghai is located on the coast, it is classified as an iodine-deficient region because of its low water concentration of iodine. In 2009, a cross-sectional survey of 7904 participants in all districts of Shanghai found that the median iodine concentration in the drinking water was only 12.8 μg L^{-1}, which is well below the standard cut-off point of 40 μg L^{-1} for iodine-deficient areas. The survey also found that iodised salt was the main source of dietary iodine in Shanghai, which accounted for 63.5% [13].

Our study found that the UIC of pregnant women in Shanghai in 2019 was 148.0 μg L^{-1}, indicating mild iodine deficiency (100–149 μg L^{-1}) according to the WHO recommendations [18]. The results are similar to those of previous Shanghai monitoring reports [13,14]. According to our findings, trimesters affect the MUIC of pregnant women. The MUIC of the pregnant women in the third trimester (139.6 μg L^{-1}) was much lower compared to the value for women in the first trimester (155.0 μg L^{-1}). Moreover, a 2018 monitoring study in the Zhejiang province of China which included 8651 pregnant women, has found that the trimester is significantly associate with low UIC, with the lowest concentration recorded in the third trimester and the highest in the first trimester [23]. This result is consistent with the results of a study of 2607 pregnant women in Chongqing, China, an iodine-sufficient area, which report that women in the first trimester had a significantly higher MUIC (189.7 μg L^{-1}) than those in third trimester (163.0 μg L^{-1}) [24]. Similarly, a study conducted in Australia found that women in the third trimester have a significantly lower MUIC than women in the first trimester (119 versus 161 μg L^{-1}) [25]. This finding may be related to the increasing of the thyroid hormone requirements of the foetus. Before 20 weeks of gestation, the foetal thyroid is not sufficiently mature to produce thyroid hormones independently. Therefore, during this period, the foetus

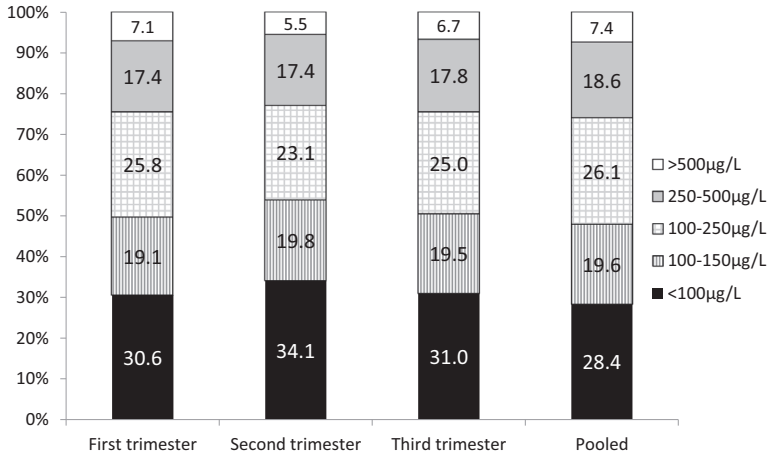

Figure 1 Proportion of urinary iodine concentrations in pregnant women (%).

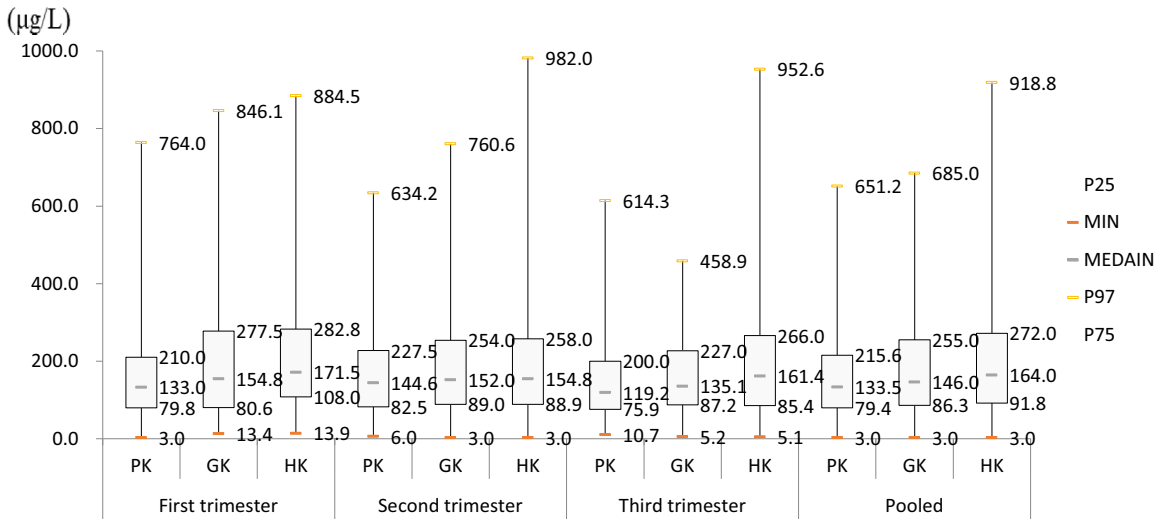

Figure 2 Distribution of urinary iodine status by the total knowledge scores of the participants. PK, poor knowledge score; GK, general knowledge score; HK, high knowledge score. *Because the maximum value is too large, which affects the appearance of the graph, the upper limit is represented by P97.

depends entirely on the maternal thyroid hormones and iodine supply [26]. After the foetal thyroid becomes mature, thyroid hormone synthesis increases and relies on the iodine supply from their mother [27], which increases the iodine requirement of pregnant women in the third trimester. Additional interventions, such as encouraging the intake of iodine-rich foods or iodine supplements, are important recommendations for pregnant women in the third trimester.

The present study found that the iodine-related dietary knowledge plays a vital role in the UIC of pregnant women, as shown in the univariate analysis and the binary logistic regression. The MUIC increased with higher knowledge scores. In our study, traditional educational status is not significant in the multivariate analysis, although the score on the iodine-related dietary knowledge suggests that the iodine-related health education is likely to be crucial with respect to preventing low UIC and cannot be replaced by traditional education. Furthermore, the 'actively learning dietary knowledge' serves as a protective factor for maintaining high UIC, thereby highlighting the importance of iodine-related health education. Nevertheless, 30.3% of the pregnant women in the present study lack iodine-related knowledge, which suggests that the iodine-related health education should be improved. A study conducted in 804 pregnant women aged 18–44 years of age in 2016 in Oslo and Norway showed that 74% of them achieved low iodine-related

Table 2 Potential factors associated with urinary iodine concentration

	β	Coefficient	95% confidence interval	*P*
Trimester				
First trimester	Reference			
Second trimester	−0.061	1.063	0.872/1.296	0.546
Third trimester	−0.245	1.278	1.048/1.559	0.016
Thyroid disease history				
No thyroid disease history	Reference			
Had thyroid disease	−0.306	–	–	0.580
Age ≥ 35 years				
No	Reference			
Yes	−0.140	–	–	0.708
Occupational status				
Mental	Reference			
Physical	−1.575	–	–	0.209
Educational status				
≤9 years	Reference			
Senior high school and college	−1.021	–	–	0.312
Bachelor's degree and above	−0.926	–	–	0.336
Family income in the past year, YUAN				
<100 000	Reference			
100 000/ 200 000	−2.887	–	–	0.089
≥200 000	−1.023	–	–	0.312
Salt groups				
Non-iodised salt	Reference			
Qualified-iodised salt	−1.569	–	–	0.210
Low-iodised salt	−1.245	–	–	0.265
Iodine-related knowledge scores				
Poor	Reference			
General	0.179	0.836	0.681/1.027	0.088
High	0.375	0.687	0.562/0.84	0.000
Having a habit of consuming iodine-rich food				
No	Reference			
Yes	0.171	0.843	0.712/0.997	0.046
Actively learn dietary knowledge				
No	Reference			
Yes	0.333	0.717	0.579/0.888	0.002
Former smoker				
No	Reference			
Yes	−0.067	–	–	0.796
Former drinker				
No	Reference			
Yes	−0.064	–	–	0.800

knowledge scores [28]. A similar result was reported in a study of the pregnant women living in Northern Ireland, where only 20% of women were aware of the potentially increased iodine requirement during pregnancy and breastfeeding period [29]. The result of a study with 2642 pregnant women in Zhejiang province, China, shows a linear upward trend in iodine-related knowledge score with higher UICs [30]. According to a 2018 cross-sectional study of pregnant women in Istanbul, nutrition knowledge score is significantly higher in the post-test (23.0) than in the pre-test (16.0) after the respondents received nutrition education ($P < 0.001$) [31]. In the follow-up to the present study, we intend to strengthen the focus on nutrition-related knowledge and enhance participants' understanding of IDD among pregnant women. Furthermore, we would aim to analyse the reasons for different iodine-related knowledge among participants.

The present study also found that the consumption rate of iodised salt is only 71.5%, whereas the consumption rate of qualified-iodised salt is only 59.4%. This may be because, even though Chinese policy has mandated that the price of non-iodised salt should not be lower than iodised salt, the merchandising of non-iodised salt has shifted from only being available via government-qualified channels to other more accessible channels, such as the Internet. On the other hand, despite the low consumption rate of qualified-iodised salt, our study found that 62.8% of pregnant women have a habit of consuming iodine-rich food. This finding may reflect the dietary habits of eating iodine-rich foods in Shanghai. The multivariate analysis also showed that having a habit of consuming iodine-rich food served as a protective factor for maintaining high UIC. Although the iodised salt is a good source of dietary iodine, it is not the only one. Therefore, we should not put excessive attention on its consumption rate; as long as pregnant women eat reasonable amounts of iodine-rich food, they can maintain their UIC at sufficient levels. Thus, it should be noted that, in the case of low usage of iodised salt, iodine-related health education is vital for pregnant population to maintain their UIC at sufficient levels.

Our research design is scientific, the sampling method is appropriate, and the detection methods are reasonable and unified. Meanwhile, the present study also has limitations. Because of the high within-person variability of a single spot urine, the single spot urine was not suitable for the analysis of individual iodine status; accordingly, we conducted a statistical conversion. Although this statistical method is applicable, it inevitably has errors. In the future, we need to strengthen our research on indicators that reflect the individual iodine status. In addition to the factors that we have corrected, there may be some other uncorrected factors that potentially influence the results.

Conclusions

In summary, pregnant women in the third trimester are at risk for mild iodine deficiency. The low

consumption of qualified-iodised salt among pregnant women is also a problem in Shanghai. The high iodine-related knowledge score, actively learning dietary knowledge, and having a habit of consuming iodine-rich food are associated with high urinary iodine excretion and have protective effects on the high UIC of pregnant women. Therefore, it is important to improve iodine-related health education for pregnant women in Shanghai. Health education should focus on third-trimester pregnant women and on guiding those who consume non-iodised salt to choose reasonable amounts of iodine-rich food.

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Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.
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All authors critically reviewed the manuscript and approved the final version submitted for publication.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with CONSORT1/STROBE2/PRISMA3 guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Iodine-related knowledge, attitudes and behaviours questionnaire.

MICRONUTRIENTS

Relationship between intake and plasma concentrations of vitamin B12 and folate in 873 adults with a physically active lifestyle: a cross-sectional study

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vitamin B12, folate, intake, plasma concentrations, physical activity.

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Abstract

Background: Vitamin B12 and folate function as co-factors in pathways used during physical activity. Physical activity may therefore increase vitamin requirements, leading to a risk of deficient plasma concentrations. We aimed to investigate the relationship between intake and plasma concentrations of vitamin B12 and folate in physically active adults, as well as identify other determinants of vitamin B12 and folate plasma concentrations.
Methods: The study population consisted of 873 adults (528 men and 345 women), aged 19–78 years, who participated in a 4-day walking event. The relationship between intake and plasma concentrations of vitamin B12 and folate was assessed using correlation and linear regression analyses. In addition, potential other determinants (sex, age, body mass index, energy intake and physical activity) of vitamin plasma concentrations were investigated.
Results: Significant positive correlations were observed between intake and plasma concentrations of vitamin B12 [Pearson's correlation coefficient = 0.15; 95% confidence interval (CI) = 0.08–0.21] and folate (Pearson's correlation coefficient = 0.18; 95% CI = 0.12–0.25). In addition to vitamin intake, sex, age and energy intake were also determinants of both vitamin B12 and folate plasma concentrations in multivariable regression models.
Conclusions: The results suggest a positive association between intake and plasma concentrations for both vitamin B12 and folate in physically active people. By contrast to our hypothesis, physical activity was not a determinant of vitamin B12 and folate plasma concentrations. However, sex, age and energy intake were found to be determinants. Thus, when studying the relationship between intake and plasma concentrations of vitamin B12 or folate, these factors should be taken into account.

Introduction

B-vitamins, such as vitamin B12 and folate, are essential for DNA synthesis and function as co-factors in various metabolic processes. Because of their role in DNA synthesis, B-vitamins are required for the synthesis of new cells, particularly red blood cells, as well as in the repair of damaged tissue. A deficiency in these micronutrients will ultimately impair cellular proliferation and function, leading to fatigue and pathophysiological conditions such as anaemia and neurological disorders [1,2,3].

A positive association between B-vitamin intake and plasma concentrations has been reported in various studies [4,5]. However, vitamin plasma concentrations depend

not only on vitamin intake, but also on vitamin uptake and vitamin metabolism. Because of their role in important pathways used during and after physical activity, it has previously been hypothesised that physical activity leads to increased requirements for B-vitamins, and/or that an increased intake of B-vitamins leads to improved recovery and performance [3]. Correspondingly, physical activity might increase the risk of deficient plasma concentrations of B-vitamins. However, evidence for these hypotheses is lacking.

Although B-vitamin intake and/or plasma concentrations have been extensively investigated in various physically active populations, in particular among (professional) athletes and military personnel [3], only a few studies have included data on both vitamin intake and vitamin plasma concentrations from the same subjects [6,7], which are necessary considerations for correctly interpreting vitamin status. In these studies, no deficient plasma concentrations of vitamin B12 and folate were observed among physically active people, nor were any differences in vitamin plasma concentrations observed between subjects with relatively low versus relatively high physical activity. However, the study populations in these studies were small ($n = 58$ [7] and $n = 76$ [6] subjects) and consisted of a specific group of athletes [6] or were limited to only women [7]. If physical exercise leads to a higher turnover of B-vitamins, this should become apparent when the relationship between intake and plasma concentrations is evaluated across a range of physical activity levels within the same study population. To our knowledge, the relationship between intake and plasma concentrations of vitamin B12 and folate has never been investigated in the general population at the same time as taking into account the level of physical activity.

In the present study, we aimed to investigate the relationship between intake and plasma concentrations of vitamin B12 and folate, in a large study population. The population consisted of Dutch adults with a physically active lifestyle, in whom the levels of physical activity covered a broad range. In addition, we aimed to identify other determinants of vitamin B12 and folate plasma concentrations, including physical activity. We hypothesised that physical activity leads to a higher turnover of vitamin B12 and folate and is an important determinant of plasma concentrations of these vitamins.

Materials and methods

Study population

The study population for the present study consisted of participants of the Nijmegen Four Days Marches 2015, an annual walking event in the Netherlands. Participants were recruited via newsletters and internet advertisements. In total, 1038 participants volunteered to participate in blood tests, as well as complete the questionnaire from the Nijmegen Exercise Study [8]. This is an ongoing online questionnaire [9], which includes a food frequency questionnaire (FFQ) and questions about supplement intake, demographic characteristics, anthropometric measures, lifestyle factors, physical activity and health status. Because we aimed to have a study population with a physically active lifestyle, 23 subjects with a physical activity level below 500 metabolic equivalent of task (MET) minutes week^{-1} were excluded. This threshold level was based on international physical activity recommendations of 500–1000 MET minutes week^{-1} [10]. In addition, 135 subjects who used vitamin B12, folate or multivitamin supplements were excluded. Finally, six subjects appeared to have extremely high plasma concentrations of vitamin B12 (>1000 pmol L^{-1}) and one subject had an extremely high plasma concentration of folate (>200 nmol L^{-1}). These values were considered abnormal; subjects with these extreme values were excluded because the cause could not be identified and these values may erroneously influence the statistical analyses. Finally, in total, 873 Nijmegen Four Days Marches participants (528 men and 345 women; aged 19–87 years) were enrolled in the present study.

The study was conducted in accordance with the Declaration of Helsinki guidelines for medical research, and all procedures involving research study participants were approved by the Medical Ethical Committee of the Radboud University Medical Center (file number: 2011/193; approval number: NL36743.091.11). Written informed consent was obtained from all subjects. Data were anonymously obtained and analysed.

Assessment of vitamin B12 and folate plasma concentrations

Venous blood samples were collected 1 or 2 days before the start of the first day of the Four Days Marches (21 July 2015), in the morning or in the afternoon. The plasma of these samples was used on the same day to determine vitamin B12 and folate concentrations. Analysis was performed at the Clinical Chemistry and Haematology Laboratory of the Gelderse Vallei Hospital. Vitamin B12 concentrations were determined on the Advia Centaur XP platform using the Acridinium Ester chemiluminescence vitamin B12 assay (Siemens, Erlangen, Germany). Folate concentrations were determined on the Vista Dimension 1500 platform using the LOCI Folate method (Siemens). Both tests were performed in accordance with the manufacturer's recommendations. Calibrators were provided as part of the assay (Siemens), and external quality control (QC) samples (Immunoassay Plus

control; Bio-Rad Laboratories, Veenendaal, the Netherlands) were analysed daily at two levels to monitor the analytical performance. Both assays performed within the acceptance criteria that were set by the manufacturer. The average imprecision of the QC analysis was 8.4% for vitamin B12 and 10.0% for folate (year-averages for 2015). In addition, the laboratory participated in an external quality assessment scheme (Dutch Foundation for Quality Assessment in Medical Laboratories; SKML), which offers six surveys per year, each containing two samples. The imprecision and accuracy were 3.8% and 98.1%, respectively, for vitamin B12; and 5.3% and 102.1%, respectively, for folate (year-averages for 2015). The methods were previously validated using external QC materials (Bio-Rad) in accordance with the EP-9 and EP-10 protocols from the Clinical Laboratory Standards Institute (CLSI).

Assessment of potential determinants of vitamin B12 and folate plasma concentrations

Data on sex, age, self-reported body height and weight, physical activity, energy intake and vitamin B12 and folate intake were obtained from the Nijmegen Exercise Study [9].

Physical activity in the past month was assessed with a short questionnaire to assess health-enhancing physical activity (SQUASH). The SQUASH has been shown to be substantially correlated with physical activity measured by accelerometry (correlation coefficient = 0.45) [11]. Using the SQUASH, the average number of minutes per week of walking, cycling, carrying out household chores, gardening, doing odd jobs and sports activities was assessed. Based on Ainsworth's compendium of physical activities [12], metabolic equivalent of task (MET) values were assigned to the specific physical activities. Subsequently, MET minutes week^{-1} were calculated by multiplying the minutes of physical activities with the accompanying MET values.

Dietary intake in the past month was assessed with a comprehensive FFQ that was validated for energy intake, macronutrients, dietary fibre and vitamins [13,14]. Pearson's correlation coefficients (crude and adjusted for both energy intake and within-person variation, respectively) for 24-h dietary recalls were 0.43 and 0.72 for vitamin B12, and 0.53 and 0.87 for folate equivalents [14]. With the FFQ the frequency of consumption of 180 food items during the previous month was assessed. Portion sizes were estimated using natural portions (e.g. one slice of bread) and commonly used household measures. Data obtained from the FFQ was converted into average daily energy and nutrient intake using data from the Dutch food composition database of 2010 [15]. The intake of folate equivalents was calculated as: μg naturally present folate + μg synthetic folic acid from fortified foods × 1.7 (+ μg folic acid from food supplements × 2.0)[16]. Note that the part of the formula in parenthesis is not applicable because we excluded subjects who used folate supplements. Possible under-reporting of dietary intake was evaluated using the Goldberg cut-off method [17,18]. The reported energy intake (EI) divided by the estimated basal metabolic rate (BMR) according to Schofield's formula [19], the EI/BMR ratio, was also calculated. This ratio was then compared with a cut-off limit of 1.55 on the group level, and with a cut-off limit of 0.87 on an individual level. The EI/BMR ratio had a mean (SD) of 1.44 (0.44), suggesting a mean underestimation of less than 10%, taking 1.55 as the cut-off limit. A total of 52 subjects (6.0%) had an EI/BMR ratio below 0.87. Sensitivity analyses comparing the results with and without these 52 subjects showed similar results; thus, subjects who possibly under-reported were not excluded.

Statistical analysis

Descriptive analyses were performed first. Because of non-normal distributions, median values and 25th to 75th percentiles of population characteristics, energy intake, intake of vitamin B12 and folate, and plasma concentrations of vitamin B12 and folate were calculated. Prevalence rates of nutrient intake below the estimated average requirement (EAR) in accordance with the recommendations of the Nordic Council (1.4 μg day^{-1} for vitamin B12 and 200 μg day^{-1} for folate equivalents) [20] were calculated using the cut-point method [21]. Prevalence rates of plasma concentrations below and above reference ranges were calculated in accordance with the reference ranges used in the Gelderse Vallei Hospital (150–600 pmol L^{-1} for vitamin B12 and 7–40 nmol L^{-1} for folate). These values are based on previous reports in the literature [22] and on results of laboratory tests in which vitamin B12 values were related to methylmalonic acid values.

The relationship between vitamin B12 and folate intake and their respective plasma concentrations was assessed using a Pearson correlation analysis and a linear regression analysis. In the regression analysis, variables also studied as potential determinants of vitamin B12 and folate plasma concentrations, in addition to vitamin B12 and folate intake, were: sex, age, body mass index (BMI), physical activity and energy intake. Univariable and multivariable regression models were fitted. In the multivariable models, potential interaction effects between the different determinants were also examined. Here, the variables age, BMI, physical activity and energy intake were first analysed categorised into quartiles. Full models containing all the main effects and all of the interaction effects were fitted. A backward stepwise selection

procedure was then used to select significant ($P < 0.05$) determinants of vitamin B12 or folate plasma concentrations. When none of the interaction terms appeared to be significant, all determinants were analysed as continuous variables. New full models containing only all of the main effects were fitted and significant determinants were selected with backward selection. Because the residuals in the models were not normally distributed, we applied a base '*e*' logarithmic transformation on the plasma concentrations of vitamin B12 and folate.

To investigate whether observed associations between determinants and vitamin plasma concentrations could be the result of a difference in vitamin intake, we compared median vitamin intake between men and women, as well as between the different quartiles of the investigated determinants. Differences between men and women were compared using a Mann–Whitney *U*-test and differences between quartiles of other determinants were compared using a Kruskal–Wallis test, followed by Dunn's post-hoc tests with Bonferroni correction.

Finally, to obtain more insight into the relationship between vitamin intake and plasma concentrations, median vitamin plasma concentrations were calculated for the different quartiles of vitamin intake. These were also compared using a Kruskal–Wallis test, followed by Dunn's post-hoc tests with Bonferroni correction.

Statistical analyses were performed with SPSS software (Version 23, IBM, Armonk, NY, USA).

Results

The study population characteristics are presented in Table 1. The median physical activity level of this physically active population was 7005 (25th to 75th percentile: 4215–9965) MET minutes week^{-1}. The median vitamin B12 intake was 4.6 (25th to 75th percentile: 3.5–6.3) μg day^{-1} and the median folate equivalents intake was 296 (25th to 75th percentile: 239–373) μg day^{-1}. The median plasma concentration of vitamin B12 was 272 (25th to 75th percentile: 221–327) pmol L^{-1} and the median plasma concentration of folate was 16.7 (25th to 75th percentile: 12.5–22.7) nmol L^{-1}. The prevalence of inadequate vitamin intake was low (0.1% for vitamin B12 and 12.0% for folate equivalents) and more than 95% of the population had vitamin plasma concentrations within the reference range.

The correlation between intake and plasma concentrations was weak for both vitamin B12 and folate. Pearson's correlation coefficient for intake and plasma concentrations of vitamin B12 was 0.15 [95% confidence interval (CI) = 0.08–0.21] ($P < 0.001$). For intake and plasma concentrations of folate, Pearson's correlation coefficient was 0.18 (95% CI = 0.12–0.25) ($P < 0.001$).

Table 1 Study population characteristics ($n = 873$)

Characteristic	Median or $n^{\dagger}$	25th to 75th percentile or %†
Sex		
Men	528	60.5
Women	345	39.5
Age (years)	63	56–67
Height (m)	1.75	1.68–1.80
Weight (kg)	76.0	67.0–84.0
Body mass index (kg m^{-2})	24.7	22.9–26.9
Physical activity (MET minutes week^{-1})	7005	4215–9965
Energy intake (kcal)	2128	1766–2512
Vitamin intake per day		
Vitamin B12 (μg)	4.6	3.5–6.3
Folate equivalents (μg)	296	239–373
Inadequate intake related to EAR		
Vitamin B12 (<1.4 μg per day)	1	0.1
Folate equivalents (<200 μg per day)	105	12.0
Vitamin plasma concentrations		
Vitamin B12 (pmol L^{-1})	272	221–327
Folate (nmol L^{-1})	16.7	12.5–22.7
Vitamin plasma concentrations related to reference ranges		
Vitamin B12		
Below reference range (<150 pmol L^{-1})	14	1.6
Within reference range (150–600 pmol L^{-1})	851	97.5
Above reference range, (>600 pmol L^{-1})	8	0.9
Folate		
Below reference range (<7 nmol L^{-1})	14	1.6
Within reference range (7–40 nmol L^{-1})	833	95.4
Above reference range (>40 nmol L^{-1})	26	3.0

Abbreviations: BMI, body mass index; EAR, estimated average requirement; MET, metabolic equivalent of task.
†Continuous variables are presented as medians and 25th to 75th percentiles; categorical variables are presented as numbers and percentages.

Table 2 presents the results of the univariable and multivariable regression analyses with vitamin B12 plasma concentrations as the dependent variable. Univariably, age and vitamin B12 intake had a positive association with plasma concentrations of vitamin B12. In the multivariable model, age and vitamin B12 intake had a positive association, whereas male sex and energy intake were negatively associated with plasma concentrations of vitamin B12. BMI and physical activity were not significantly associated with plasma concentrations of vitamin B12 in the multivariable model. The explained variance (r^2) of the multivariable model was 0.050.

Table 2 Associations between investigated determinants and vitamin B12 plasma concentrations

Characteristic	B (95% CI)
Univariable	
Sex (men)	−0.033 (−0.074 to 0.007)
Age (years)	0.002* (0.000 to 0.004)
BMI (kg m^{-1})	−0.003 (−0.009 to 0.003)
Physical activity (MET minutes $week^{-1}$)	0.000 (−0.000 to 0.000)
Energy intake per day (kcal)	0.000 (−0.000 to 0.000)
Vitamin B12 intake per day (μg)	0.018* (0.011 to 0.026)
Multivariable†	
Intercept	5.422* (5.291 to 5.554)
Sex (men)	−0.063* (−0.106 to −0.020)
Age (years)	0.003* (0.001 to 0.005)
Energy intake per day (kcal)	−0.000* (−0.000 to −0.000)
Vitamin B12 intake per day (μg)	0.025* (0.017 to 0.034)

The beta represents the difference in the log *e* transformed predicted value of vitamin B12 plasma concentrations in pmol L^{-1} for 1 μg increase of vitamin B12 intake. Thus, a beta of 0.018 for vitamin B12 intake means that, for a 1 μg increase of vitamin B12 intake, the vitamin B12 plasma concentration increases by exp(0.018) = 1.018 nmol L^{-1}, which corresponds to an increase of 1.8%.

Abbreviations: BMI body mass index; CI, confidence interval; MET metabolic equivalent of task.

*Significant determinant of vitamin B12 plasma concentrations ($P < 0.05$).

†The final multivariable model after backward selection of significant determinants is presented.

Table 3 Associations between investigated determinants and folate plasma concentrations

Characteristic	B (95% CI)
Univariable	
Sex (men)	−0.084* (−0.144 to −0.024)
Age (years)	0.007* (0.005 to 0.010)
BMI (kg m^{-1})	−0.014* (−0.023 to −0.005)
Physical activity (MET minutes $week^{-1}$)	0.000 (−0.000 to 0.000)
Energy intake per day (kcal)	−0.000 (−0.000 to 0.000)
Folate equivalents intake per day (μg)	0.001* (0.001 to 0.001)
Multivariable†	
Intercept	2.293* (2.106 to 2.481)
Sex (men)	−0.120* (−0.182 to −0.058)
Age (years)	0.008* (0.005 to 0.010)
Energy intake per day (kcal)	0.000* (0.000 to −0.000)
Folate equivalents intake per day (μg)	0.001* (0.001 to 0.002)

The beta represents the difference in the log-e transformed predicted value of folate plasma concentrations in nmol L^{-1} for 1 μg increase of folate equivalents intake. Thus, a beta of 0.001 for folate equivalents intake means that, for a 1 μg increase of folate equivalents intake, the folate plasma concentration increases by exp(0.001) = 1.001 nmol L^{-1}, which corresponds to an increase of 0.1%.

Abbreviations: BMI, body mass index; CI, confidence interval; MET, metabolic equivalent of task.

*Significant determinant of folate plasma concentrations ($P < 0.05$).

†The final multivariable model after backward selection of significant determinants is presented.

The results of the univariable and multivariable regression analyses, with folate plasma concentrations as dependent variable, are presented in Table 3. Univariably, age and folate equivalents intake had a positive association with plasma concentrations of folate, whereas male sex and BMI had a negative association. In the multivariable model, age, energy intake and folate equivalents intake were positively associated, whereas male sex was negatively associated with plasma concentrations of folate. BMI and physical activity were not significantly associated with plasma concentrations of folate in the multivariable model. The r^2 of the multivariable model was 0.116.

Table 4 presents the vitamin intake in men and women separately, and in different quartiles of the investigated determinants of vitamin plasma concentrations. Men had a higher intake of both vitamin B12 and folate equivalents compared to women, older subjects had a higher folate equivalents intake compared to younger subjects, and a higher energy intake was accompanied by a higher intake of both vitamin B12 and folate equivalents.

Table 5 presents the vitamin plasma concentrations in different quartiles of vitamin intake. In general, a higher vitamin intake was accompanied by higher vitamin plasma concentrations.

Discussion

The present study aimed to assess the relationship between intake and plasma concentrations of vitamin B12 and folate, as well as identify determinants of vitamin B12 and folate plasma concentrations, in a large population of adults with a physically active lifestyle. We observed a positive association between intake and plasma concentrations for both vitamin B12 and folate. By contrast to our hypothesis, physical activity was not a determinant of vitamin B12 and folate plasma concentrations. On the other hand, sex, age and energy intake were found to be determinants of both vitamin B12 and folate plasma concentrations in the multivariable regression models.

The observed positive association between vitamin B12 and folate equivalents intake and their respective plasma concentrations is in agreement with observations in previous studies [4,5]. However, it should be noted that these studies have been conducted in various study populations, and are not limited to physically active populations. We observed weak correlation coefficients for vitamin intake and plasma concentrations (0.15 for vitamin B12 and 0.18 for folate), which is also in agreement with the

Table 4 Median daily intake of vitamin B12 and folate equivalents per sex and per quartile of investigated determinant of vitamin plasma concentrations

Quartile/Sex	Sex	Age	BMI	Physical activity	Energy intake
	Vitamin B12 (μg)	*Vitamin B12 (μg)*	*Vitamin B12 (μg)*	*Vitamin B12 (μg)*	*Vitamin B12 (μg)*
1 Men	4.9 (3.7–6.7)*	4.6 (3.5–6.1)	4.7 (3.4–6.2)	4.3 (3.4–5.9)	3.3 (2.6–4.6)*[2,3,4]
2 Women	4.2 (3.3–5.6)*	4.6 (3.4–6.5)	4.6 (3.4–6.1)	4.6 (3.5–6.1)	4.5 (3.6–6.1)*[1,4]
3		4.6 (3.5–6.1)	4.8 (3.6–6.7)	4.9 (3.8–6.7)	4.9 (4.0–6.2)*[1,4]
4		4.7 (3.6–6.9)	4.5 (3.5–6.0)	4.5 (3.4–6.4)	6.1 (4.5–8.4)*[1,2,3]
	Folate equivalents (μg)	*Folate equivalents (μg)*	*Folate equivalents (μg)*	*Folate equivalents (μg)*	*Folate equivalents (μg)*
1 Men	304 (244–388)*	278 (227–346)*[4]	294 (251–387)	298 (228–361)	231 (185–281)*[2,3,4]
2 Women	286 (234–357)*	296 (243–369)	307 (238–386)	294 (239–346)	278 (237–344)*[1,3,4]
3		301 (238–385)	297 (242–362)	301 (241–391)	312 (260–371)*[1,2,4]
4		307 (254–397)*[1]	283 (231–363)	293 (245–378)	388 (314–463)*[1,2,,3]

Values of vitamin intake are presented as the median (25th to 75th percentile).
Abbreviation: BMI, body mass index.
*Significant difference between men and women and between quartiles of investigated determinants ($P < 0.05$). The number after the asterisk (*) denotes the quartile from which the given quartile is significantly different.

Table 5 Median plasma concentrations of vitamin B12 and folate per quartile of vitamin intake

Quartile of vitamin intake	Vitamin intake per day	Vitamin plasma concentrations
	Vitamin B12 (μg)	*Vitamin B12 (pmol L^{-1})*
1	<3.5	260 (212–307)*[3,4]
2	≥3.5 and <4.6	251 (215–312)*[3,4]
3	≥4.6 and <6.3	281 (237–333)*[1,2]
4	≥6.3	290 (235–350)*[1,2]
	Folate equivalents (μg)	*Folate (nmol L^{-1})*
1	<239	14.7 (10.9–19.5)*[3,4]
2	≥239 and <296	15.0 (12.5–21.2)*[3,4]
3	≥296 and <373	17.8 (13.2–24.7)*[1,2]
4	≥373	19.5 (14.6–25.0)*[1,2]

Vitamin plasma concentrations are presented as the median (25th to 75th percentile).
*Significant difference between quartiles of vitamin intake ($P < 0.05$). The number after the asterisk (*) denotes the quartile from which the given quartile is significantly different.

literature. For intake and plasma concentrations of vitamin B12, correlation coefficients ranging from 0.06 to 0.21 have been reported [23,24,25]. In a review of 17 studies on the validity of intake and measurement of folate, correlation coefficients ranged from 0.05 to 0.54 [26]. An explanation for the broad range in correlation coefficients for folate is that the correlations were considerably higher in studies where supplement use was taken into account. Also, stronger correlations with plasma folate concentrations have been reported for folic acid from fortified foods compared to naturally occurring food folate [27]. This is likely a consequence of the higher bioavailability of synthetic folic acid than that of natural food folate [28]. To adjust for these differences in bioavailability, it is recommended to express folate intake as dietary folate equivalents, which we did in the present study.

Male sex showed a significant negative association with both vitamin B12 and folate plasma concentrations in the multivariable models, whereas, in the univariable models, the negative association was only significant for folate plasma concentrations. Lower concentrations of vitamin B12 in men compared to women have been reported in certain studies [29,30,31], although not in all [32]. Likewise, for folate concentrations, lower concentrations have been observed in men compared to women [30,31], although not in all studies [29,32]. It is worth noting that most of these studies were conducted in elderly people. In a study among African Americans aged 21–94 years, lower vitamin B12 and folate concentrations in men compared to women were observed across different age groups, except for the 21–34-year-old age group [33]. The negative association of male sex with vitamin plasma concentrations cannot be explained by differences in vitamin intake between men and women. Moreover, vitamin intake in men was even higher than in women. An explanation for these opposing observations could be that men have a larger body size and therefore a larger distribution volume, or that there is a difference in lean body mass between men and women [34]. Another possible explanation for the observed association between sex and vitamin plasma concentrations could be hormonal effects. Variations in folate concentrations during different phases of the ovarian cycle have been reported [35]. Also, lower concentrations of vitamin B12 in women using oral contraceptives compared to non-users have been reported [36,37]. The mechanism by which oral contraceptives reduce vitamin B12 concentrations is unclear, although a decreasing effect on vitamin B12 binding capacity in

serum has been suggested as an explanation [38]. Although the results of these studies cannot explain the association between sex and vitamin plasma concentrations in the present study, these studies indicate that gonadal steroids might influence vitamin plasma concentrations.

We observed a significant positive association with age for both vitamin B12 and folate plasma concentrations in the univariable and in the multivariable models. This is in agreement with the study among African Americans [33], in which a general increase in vitamin B12 and folate concentrations was seen with older age. An explanation for the positive association between age and folate plasma concentrations could be a higher folate equivalents intake with increasing age. However, a higher vitamin B12 intake with increasing age was not observed, and so this cannot explain the positive association between age and vitamin B12 plasma concentrations.

In the multivariable models, energy intake showed a significant negative association with vitamin B12 plasma concentrations, and a significant positive association with folate plasma concentrations. However, in the univariable models, opposite associations were observed: energy intake showed a non-significant positive association with vitamin B12 plasma concentrations and a significant negative association with folate plasma concentrations. Positive associations could be explained by an increased vitamin intake with a higher energy intake in the current study. However, this contradicts the observed negative associations. An explanation for these findings could be that the observed associations are very small and that any associations in the multivariable models are also influenced by the effects of other determinants in the multivariable models.

Although it is often assumed that physical activity leads to increased vitamin requirement, evidence for this hypothesis is lacking [39]. Both in the univariable and in the multivariable models, we observed no association between physical activity and vitamin plasma concentrations. This observation is in agreement with studies in which no differences were observed in vitamin B12 and folate concentrations between groups with relatively low and relatively high physical activity [6,7]. However, in one of these studies, in which only women were included, a higher intake of vitamin B12 and folate in the high physical activity group was observed [7], which might explain the absence of differences in vitamin concentrations. In the women-only study, it could be that differences in vitamin concentrations would have been observed between the two groups should vitamin intake have been similar in the two groups. However, in the other study in which no differences in vitamin concentrations were observed, folate intake in men and vitamin B12 intake in men and women were similar in groups with both low and high physical activity [6]. In the present study, vitamin intake was also not different between different quartiles of physical activity; thus, a higher vitamin intake in subjects with a higher physical activity level cannot explain the lack of an association between physical activity and vitamin plasma concentrations.

The results of the present study suggest that sex, age and energy intake should be taken into account when studying the relationship between intake and plasma concentrations of vitamin B12 and folate. By contrast to our hypothesis, we did not find evidence that physical activity influences plasma concentrations of these vitamins. This suggests that it is not necessary to take into account physical activity level when studying the relationship between intake and plasma concentrations of vitamin B12 and folate.

In this physically active study population who did not use vitamin supplements, the intake of both vitamin B12 and folate equivalents can be regarded as adequate, considering that, for both vitamins, the median intake was higher than the EAR and only a small percentage of the population had an intake below the EAR. However, these findings must be viewed carefully because an FFQ is not the best method to evaluate adequacy of nutrient intake. Plasma concentrations of vitamin B12 and folate can also be regarded as generally sufficient, considering that more than 95% of the population had plasma concentrations within the reference range, and only 1.6% had deficient plasma concentrations. The results of the present study may therefore also suggest that people who are at least moderately physically active do not need a higher vitamin intake than persons with low physical activity to maintain sufficient plasma concentrations. In addition, it is likely unnecessary for physically active people to use vitamin supplements when their vitamin intake from foods meets the EAR. However, further intervention studies designed to specifically evaluate dietary requirements are necessary to experimentally confirm these suggestions.

To our knowledge, this is the first study in which the relationship between intake and plasma concentrations of vitamin B12 and folate has been investigated in a large study population, comprising both men and women over a broad age range, with varying levels of physical activity. A limitation of the study is related to the self-reporting methods for the assessment of dietary intake and physical activity. Although we used validated questionnaires that are frequently used, all self-reporting methods are prone to several types of error such as recall bias or the tendency to provide socially desirable answers [40,41]. A specific limitation of an FFQ is that single foods are grouped into groups of food items, where the variation of reported intake may be underestimated. This results in a

smaller distribution of nutrient intake, and consequently an underestimation of the prevalence rate of inadequate intake. Therefore, reported prevalence rates of inadequate intake should be interpreted with caution. However, the median values and prevalence rates of inadequate intake observed in the present study are in agreement with values and prevalence rates observed in the Dutch National Food Consumption Survey [42] and a study among Dutch elite and sub-elite athletes [43]. In these studies, dietary intake was assessed with 24-h dietary recalls, which have a higher precision in estimating the distribution of dietary intake [44]. Also, evaluation of energy intake using the Goldberg cut-off method indicated that under-reporting was limited in the present study. Another limitation is that folate was analysed in plasma, which is known to be sensitive for transient changes in folate intake. Red blood cell folate is considered to be a more robust indicator of folate status than plasma folate because red blood cell folate reflects folate status over the last 3–4 months [2,45]. Synthetic folic acid has a higher bioavailability than natural food folate [28], which may therefore be a confounding factor in plasma folate analysis. It is worthwhile noting that fortification is limited in the Netherlands (maximum 100 μg of folic acid per 100 kcal) and that supplement users were excluded in the present study.

In conclusion, we observed a positive association between intake and plasma concentrations for both vitamin B12 and folate in people with a physically active lifestyle. By contrast to our hypothesis, physical activity was not a determinant of vitamin B12 and folate plasma concentrations. However, sex, age and energy intake were found to be determinants. Thus, when investigating the relationship between intake and plasma concentrations of vitamin B12 or folate, sex, age and energy intake should be taken into account.

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Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with STROBE guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.
This study was financially supported by the EAT2MOVE project and a grant from the Province of Gelderland, proposal PS2014-49. AMB, MGJB and JMTKG designed the study. JdV was responsible for the FFQ. DSMtH and MTEH provided the data. AMB analysed the data. All authors interpreted the results. AMB wrote the paper. All authors critically reviewed and approved the final manuscript submitted for publication. All authors are responsible for the final content.

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FATTY ACIDS

Examining the association between serum free fatty acids and blood levels of testosterone

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Keywords
dietary fat, fat, obesity, serum, serum free fatty acids, testosterone.

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Abstract

Background: Multiple studies have uncovered the effects that ingested fat has on human blood levels of testosterone. Yet, few reports have discussed the effect of circulating serum free fatty acids (FFAs). The present study aimed to explore the relationship between serum free fatty acids and blood levels of testosterone.
Methods: In total, 5719 adults were pooled from the database of the National Health and Nutrition Examination Survey (NHANES) from 2011 to 2012. Based on multivariable-linear regression models, we employed a total of 30 FFAs to interpret the relationship of FFAs with blood levels of testosterone. Two models with covariate adjustments were designated for further evaluation and analysis.
Results: Capric acid [$\beta = -0.014$, 95% confidence interval (CI) = −0.023, −0.004, $P = 0.005$], myristic acid ($\beta = -0.001$, 95% CI = −0.001, 0.000, $P \leq 0.001$), pentadecanoic acid ($\beta = -0.013$, 95% CI = −0.018, −0.008, $P \leq 0.001$), margaric acid ($\beta = -0.011$, 95% CI = −0.017, −0.005, $P \leq 0.001$) and alpha-linolenic acid ($\beta = -0.001$, 95% CI = −0.002, 0.000, $P = 0.004$) in the fully adjusted model were significantly negatively correlated with the testosterone level inh obese men. In the fully adjusted model for the female analysis, myristic acid, pentadecanoic acid, palmitic acid, margaric acid, stearic acid, myristoleic acid, oleic acid, nervonic acid and alpha-linolenic acid were found significantly associated with the testosterone level.
Conclusions: Our findings indicate a significant negative correlation between serum FFAs and blood levels of testosterone. Furthermore, we reveal the essentiality of serum FFAs and their potential effects on the reduction of testosterone levels.

Introduction

Free fatty acids (FFAs) circulate in the plasma via transport proteins and are products of the lipolysis of reservoir triglycerides [(1)]. Fatty acids are characterised according to the number of double bonds, the position of the double bond and the length of the chain. Saturated fatty acids have no double bonds, whereas unsaturated fatty acids present at least one double bond, hence the use of the terms monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) [(2)].

FFAs can be taken up through dietary ingestion [(3)]. FFA uptake by cells provides an essential energy resource and is critical in the activation of receptors and the expression of genes [(4)]. FFAs act as signalling molecules that activate free fatty acid receptors (FFARs), which can modulate biological and physiological functions. Butyric acid, for example, is a FFA that affects the expression of genes involved in the regulation of cell proliferation, differentiation and apoptosis [(5)]. It also stimulates the transcription of the calcitonin gene in cultured human medullary thyroid carcinoma [(5)]. Dietary fish oil *n*-3

PUFAs are credited with cardiovascular protective effects by reducing circulating very-low-density lipoprotein and chylomicrons in patients with hypertriglyceridaemia [5]. It has been postulated that this protective effect of PUFAs is the result of an inhibition of gene transcription and/or modifications in mRNA maturation [6]. FFARs activated by FFAs can cause several physiological effects. These include the facilitation of insulin and incretin hormone secretion, adipocyte differentiation, neuronal responses, taste preference and pain control [4]. Particularly in insulin secretion, FFAR1 is shown to be highly expressed in pancreatic insulin-producing β cells. Thus, the overexpression of FFAR1 improves insulin secretion in obese individuals [4]. Conversely, inhibited glucose-stimulated insulin secretion is observed in FFAR3-overexpressing islets [4]. A review by Boden hypothesises that FFA-induced insulin oversecretion is unable to compensate for FFA-induced insulin resistance in obese subjects genetically predisposed to developing type 2 diabetes [7]. FFAs induce insulin resistance in muscle via the hexosamine biosynthetic pathway by increasing reactive oxygen species, comprising reactive molecules that cause oxidative damage [8]. Moreover, studies report that FFAs are strongly linked to inflammation [4,9]. Taken together, FFAs possess properties that can regulate events such as insulin resistance and obesity [9-11], cardiovascular diseases [12,13] and metabolic syndromes [11].

Many reports have discussed the complications related to a higher fat diet. An increasing number of studies have reported an inverse association between the dietary intake of fats and gonadal functions. Jensen *et al.* [14] found a dose-dependent relationship between increased dietary intake of saturated fat and total sperm count in young Danish men. Minguez-Alarcón *et al.* [15] conducted a study on young Spanish men and found an inverse association between MUFA intake and testosterone levels. A recent review by Collodel *et al.* [16] suggested a possible linkage between ingested fats and male infertility. Although an increasing dietary intake of fats is related to adverse health events, few studies have examined the direct impact of circulating serum FFAs on the human body. The present research aimed to identify such an impact by examining the relationship between serum FFAs and blood levels of testosterone in a nationally representative sample of US adults.

Materials and methods

Ethics statement

All data were obtained from the National Health and Nutrition Examination Survey (NHANES). NHANES is a cross-sectional survey designed to collect health and nutritional information of non-institutionalised citizens in the USA and was performed by the National Center for Health Statistics (NCHS) [17,18]. The survey was conducted annually and consisted of demographic, clinical, behavioural, dietary, social and laboratory data. All NHANES study customs were approved by the NCHS Institutional Review Board. The NHANES administration obtained informed consent from eligible subjects before initiating interviews and data collection.

Study sample

The study sample was collected from the NHANES database from 2011 to 2012. The survey was conducted with an initial household interview followed by physical examination at the Mobile Examination Center (MEC). Information regarding age, sex, race and medical history was collected by a trained examiner during the interview session. The sample size of the study consisted of 5719 men and women. We included all participants who had proper identity and were aged 18 years and over and excluded participants with inadequate data or interview responses. Participants who were 60 years old or older were categorised as the elderly group. Participants whose body mass index (BMI) was 30 kg m^{-2} or over were categorised as the obese group. The demographic information is displayed in Table 1.

Measurement of serum free fatty acids

With mineral acids and bases, esterified fatty acids can be hydrolysed from triglycerides, phospholipids and cholesteryl esters in the presence of heat. The FFAs were first extracted from 100 μL of serum and derivatised to pentafluorobenzyl esters with pentafluorobenzyl bromide along with the presence of triethylamine [19]. The reaction mixtures were distributed into capillary gas chromatography columns and quantified by electron capture negative-ion mass spectrometry. Finally, the data were calibrated by comparing the unknown result with that of the calibrator solution.

Measurement of total testosterone

The National Institute for Standards and Technology method was employed to extract and isolate testosterone from the serum [20]. These isolated testosterone samples were then inspected via isotope dilution liquid chromatography tandem mass spectrometry for quantitation [21].

Measurement: covariates

Demographic data such as race/ethnicity, sex, age and medical history were acquired from self-reported data.

Table 1 Demographics

Characteristics of study participants	Study participants			
	Men (n = 2820)	Women (n = 2899)	Total (n = 5719)	P-value
*Continuous variables**				
Age at screening	47.87 (18.40)	48.35 (18.20)	48.11 (18.29)	0.199
Glucose (mg dL^{-1})	104.59 (41.24)	100.33 (38.39)	102.45 (39.89)	0.005
Creatinine (mg dL^{-1})	1.02 (0.43)	0.79 (0.37)	0.91 (0.42)	0.041
Cholesterol (mg dL^{-1})	185.46 (41.30)	194.35 (41.83)	189.93 (41.80)	0.814
AST (U L^{-1})	27.83 (19.81)	23.52 (13.07)	25.66 (16.91)	<0.001
BMI (kg m^{-2})	28.14 (6.01)	29.20 (7.61)	28.68 (6.89)	<0.001
Capric acid (C10:0) (μmol L^{-1})	3.31 (6.43)	2.26 (5.27)	2.96 (5.88)	<0.001
Lauric acid (C12:0) (μmol L^{-1})	14.33 (22.40)	13.33(32.58)	13.83 (27.99)	0.255
Myristic acid (14:0) (μmol L^{-1})	148.40 (130.54)	129.96 (98.89)	139.13 (116.06)	<0.001
Pentadecanoic acid (C15:0) (μmol L^{-1})	24.40 (13.00)	24.18 (10.99)	24.29 (12.03)	0.005
Palmitic acid (16:0) (μmol L^{-1})	3057.89 (1331.94)	2921.80 (1099.37)	2989.44 (1222.17)	<0.001
Margaric acid (C17:0) (μmol L^{-1})	32.53 (11.63)	31.63 (9.50)	32.08 (10.61)	<0.001
Stearic acid (18:0) (μmol L^{-1})	707.97 (245.52)	707.09 (197.52)	707.52 (222.63)	<0.001
Arachidic acid (20:0) (μmol L^{-1})	23.15 (6.40)	25.01 (6.01)	24.08 (6.27)	0.380
Myristoleic acid (14:1*n*-5) (μmol L^{-1})	9.20 (10.97)	8.45 (10.28)	8.82 (10.63)	0.002
Palmitoleic acid (16:1*n*-7) (μmol L^{-1})	252.97 (204.11)	254.32 (213.79)	253.65 (208.99)	0.127
Cis-Vaccenic acid (18:1*n*-7) (μmol L^{-1})	157.50 (66.44)	153.35 (69.49)	155.41 (68.01)	0.303
Oleic acid (18:1*n*-9) (μmol L^{-1})	2373.85 (1182.57)	2161.43 (1039.50)	2267.01 (1117.74)	<0.001
Nervonic acid (24:1*n*-9) (μmol L^{-1})	83.13 (20.21)	93.66 (24.83)	88.44 (23.25)	<0.001
Linoleic acid (18:2*n*-6) (μmol L^{-1})	3780.09 (1126.92)	3816.24 (930.88)	3798.29 (1032.85)	0.001
Alpha-linolenic acid (18:3*n*-3) (μmol L^{-1})	97.70 (69.55)	91.44 (53.59)	94.55 (62.10)	<0.001
Gamma-linolenic acid (C18:3*n*-6) (μmol L^{-1})	60.85 (34.82)	59.52 (34.41)	60.18 (34.61)	0.711
Stearidonic acid (C18:4*n*-3) (μmol L^{-1})	4.08 (3.79)	3.94 (4.42)	4.01 (4.13)	0.255
Eicosadienoic acid (20:2*n*-6) (μmol L^{-1})	24.15 (9.84)	24.04 (8.88)	24.10 (9.37)	0.029
Homo-gamma-linolenic acid (20:3*n*-6) (μmol L^{-1})	155.79 (59.40)	163.62 (61.25)	159.72 (60.45)	0.150
Docosatetraenoic acid (22:4*n*-6) (μmol L^{-1})	27.54 (11.11)	26.21 (12.16)	26.87 (11.67)	0.645
Docosapentaenoic acid (22:5*n*-6) (μmol L^{-1})	20.25 (8.59)	21.42 (10.56)	20.84 (9.65)	0.002
Tricosanoic acid (C23:0) (μmol L^{-1})	26.82 (6.52)	30.64 (7.46)	28.74 (7.26)	<0.001
Lignoceric acid (24:0) (μmol L^{-1})	56.48 (13.85)	57.22 (14.19)	56.85 (14.02)	0.169
Eicosenoic acid (20:1*n*-9) (μmol L^{-1})	15.72 (9.04)	14.18 (7.00)	14.95 (8.12)	<0.001
Eicosatrienoic acid (C20:3*n*-9) (μmol L^{-1})	8.28 (5.63)	7.71 (5.48)	8.00 (5.56)	0.105
Arachidonic acid (20:4*n*-6) (μmol L^{-1})	866.44 (256.28)	895.57 (270.119)	881.10 (263.68)	0.217
Eicosapentaenoic acid (20:5*n*-3) (μmol L^{-1})	65.68 (55.21)	72.77 (75.18)	69.25 (66.10)	<0.001
Docosapentaenoic acid (22:5*n*-3) (μmol L^{-1})	53.22 (20.98)	51.77 (20.90)	52.49 (20.95)	0.549
Docosahexaenoic acid (22:6*n*-3) (μmol L^{-1})	159.54 (80.54)	182.89 (95.12)	171.30 (88.93)	<0.001
Docosanoic acid (22:0) (μmol L^{-1})	63.97 (15.93)	69.55 (17.12)	66.79 (16.77)	0.002
Categorical variables†				
Race/ethnicity	299 (10.6)	266 (9.2)	565 (9.9)	0.036
Congestive heart failure	90 (3.2)	97 (3.4)	187 (3.4)	0.769
Coronary heart disease	128 (4.5)	68 (2.4)	196 (3.5)	<0.001
Angina/angina pectoris	63 (2.2)	66 (2.3)	129 (2.3)	0.646
Heart attack	123 (4.4)	80 (2.8)	203 (3.7)	0.002
Smoking	1451 (51.5)	918 (32.6)	2369 (42.6)	<0.001

AST, aspartate aminotransferase; BMI, body mass index.

*Values are expressed as the mean (SD).

†Values in the categorical variables are expressed as n (%).

Cigarette use was assessed by asking the question 'Have you ever smoked cigarettes?'. Medical histories were based on the diagnosis of past medical histories. Laboratory data such as BMI, aspartate aminotransferase (AST), serum glucose, serum FFAs, creatinine and cholesterol were also analysed. Specific details of the measurement were obtained from the NHANES documentation [18].

Statistical analysis

The present study utilised SPSS, version 18.0 (SPSS, Inc., Chicago, IL, USA) to perform the statistical analysis. A chi-squared test and Wilcoxon rank-sum test were applied to categorical variables and continuous variables, respectively. Based on multivariable-linear regression models, we interpreted the association between serum FFAs and blood levels of testosterone. Two models with individual covariate adjustments were investigated using extended-model linear regressions. We included an unadjusted model and a fully adjusted model that consisted of covariates such as age, race/ethnicity, AST, BMI, serum glucose, creatinine, medical histories of congestive heart failure, coronary heart disease, angina/angina disease and heart attack.

Results

Study sample characteristics

The study participants included 2820 men and 2899 women, giving a total of 5719 subjects (Table 1). The mean (SD) age and BMI for the participants were 48.11 (18.29) years old and 28.68 (6.89), respectively. The study explored 30 serum FFAs and their relationship with the study participants. Among these FFAs, linoleic acid (18:3*n*-3) had the highest serum concentration [3798.29 (1032.85) μmol L^{-1}], whereas capric acid (C10:0) had the lowest serum concentration [2.96 (5.88) μmol L^{-1}].

Relationship between serum free fatty acid and testosterone levels

The relationship between serum FFAs and testosterone levels is illustrated in Fig. 1 for men and in Fig. 2 for women. FFAs that presented a negative correlation with the testosterone level of men in the unadjusted model were capric acid, lauric acid, pentadecanoic acid, margaric acid, myristoleic acid, alpha-linolenic acid, gamma-linolenic acid, stearidonic acid, homo-gamma-linolenic acid, docosatetraenoic acid and docosapentaenoic acid (22:5*n*-6) (Fig. 1a). After covariate adjustments, docosapentaenoic acid (22:5*n*-3) appeared to be negatively associated with testosterone levels (Fig. 1b). In the unadjusted model of the analysis for women, pentadecanoic acid, margaric acid, stearidonic acid and docosapentaenoic acid were negatively correlated with testosterone levels (Fig. 2a). The direction of the association remained similar after covariate adjustment (Fig. 2b).

FFAs of statistical significance from Table 1 were included in Table 2 and analysed with the testosterone level of non-obese individuals. Significantly associated FFAs for men in Table 2 included myristic acid, pentadecanoic acid, palmitic acid, margaric acid, stearic acid, myristoleic acid, oleic acid, alpha-linolenic acid and docosanoic acid in the fully adjusted models ($P < 0.05$). Statistically significant FFAs in Table 2 either indicated a neutral or negative correlation with the testosterone level for men. Nervonic acid, docosapentaenoic acid and docosahexaenoic acid in the same analysis and model for females showed a positive association. Conversely, Table 3 displays the association between selected FFAs from Table 1 and the level of testosterone in obese individuals. Capric acid [β = −0.014, 95% confidence interval (CI) = −0.023, −0.004, $P = 0.005$], myristic acid (β = −0.001, 95% CI = −0.001, 0.000, $P \leq 0.001$), pentadecanoic acid (β = −0.013, 95% CI = −0.018, −0.008, $P \leq 0.001$), margaric acid (β = −0.011, 95% CI = −0.017, −0.005, $P \leq 0.001$) and alpha-linolenic acid (β = −0.001, 95% CI = −0.002, 0.000, $P = 0.004$) in the fully adjusted model were significantly negatively correlated with the testosterone level of obese men, whereas palmitic acid (β = 0.000, 95% CI = 0.000, 0.000, $P \leq 0.001$) and stearic acid (β = 0.000, 95% CI = −0.001, −0.000, $P = 0.004$) displayed neutral effects. Myristic acid, pentadecanoic acid, palmitic acid, margaric acid, stearic acid, myristoleic acid, oleic acid, nervonic acid and alpha-linolenic acid in the fully adjusted model in the female analysis revealed statistical significance, with only nervonic acid having a positive association.

In the Supporting information, Tables S1 and S2 include age in the analysis of FFAs and testosterone level correlations. Table S1 summarises the correlation for subjects under 60 years old. It was found that all FFAs in the fully adjusted model except capric acid ($P = 0.153$) displayed a significance that either indicated a neutral or negative correlation between FFAs and testosterone levels in men. Nervonic acid showed statistical significance in the female fully adjusted model ($P \leq 0.001$). In the Supporting information, Table S2 shows the correlation for subjects aged 60 years or over. In men, capric acid, myristic acid, pentadecanoic acid, palmitic acid, margaric acid, myristoleic acid, oleic acid, alpha-linolenic acid and eicosapentaenoic acid showed statistical significance. For females, pentadecanoic acid, margaric acid, linolenic acid and alpha-linolenic acid were found to be significantly correlated. Evidently, there were neutral and negative correlations between FFAs and testosterone levels for subjects over 60 years old.

Discussion

A significant association between serum FFAs and the testosterone level of men was discovered in a nationally representative sample of the adult population in the USA.

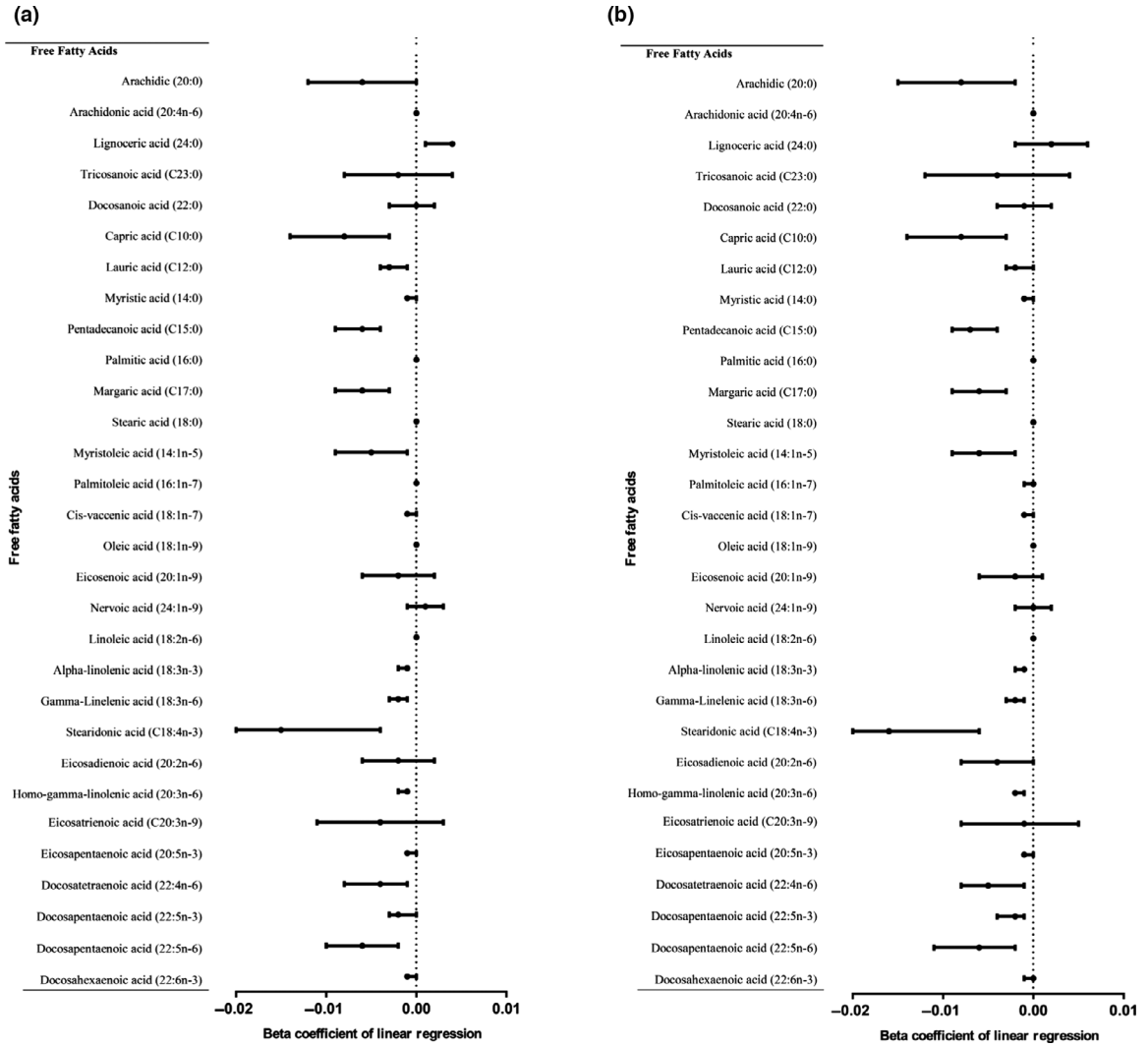

Figure 1 (a) Association between free fatty acids (FFAs) and testosterone levels for men in an unadjusted model. (b) Association between FFAs and testosterone levels for men in a fully adjusted model. Covariate adjustment for the fully adjusted model: age, race/ethnicity, aspartate aminotransferase, body mass index, serum glucose, creatinine, congestive heart failure, coronary heart disease, angina/angina disease, heart attack.

The present study emphasised the measurement of serum FFAs and explored their potential relationships with testosterone levels. We noted a negative correlation between serum FFAs and serum blood levels of testosterone throughout multiple analyses. Although existing studies have evaluated the influence of fat ingested, our study stressed the significance of serum measurement. Notably, our findings provided the relationship between circulating serum FFAs and serum blood levels of testosterone.

Our study revealed that an elevation in serum FFAs was related to lower testosterone levels. The mechanism behind this relationship is unclear. However, we speculate that obesity is an important contributing factor. Serum FFAs are related to obesity [22,23]. Multiple studies have claimed that elevated serum FFAs are found in most obese individuals as a result of their effect on insulin resistance [9,24,25]. Another study confirmed the influence of FFAs on gene activation and the subsequent development of obesity [26]. Although the relationship between obesity and the testosterone concentration is commonly acknowledged as bidirectional [27], rising evidence favors obesity as the cause [28]. One example would be the regulation of hormones. Jones *et al.* [27] expanded on the theory that excess adipose tissue and the adipose-derived hormone known as leptin could influence the production of testosterone. Gonadotrophin-releasing hormone (GnRH) neurones in the hypothalamus are responsible

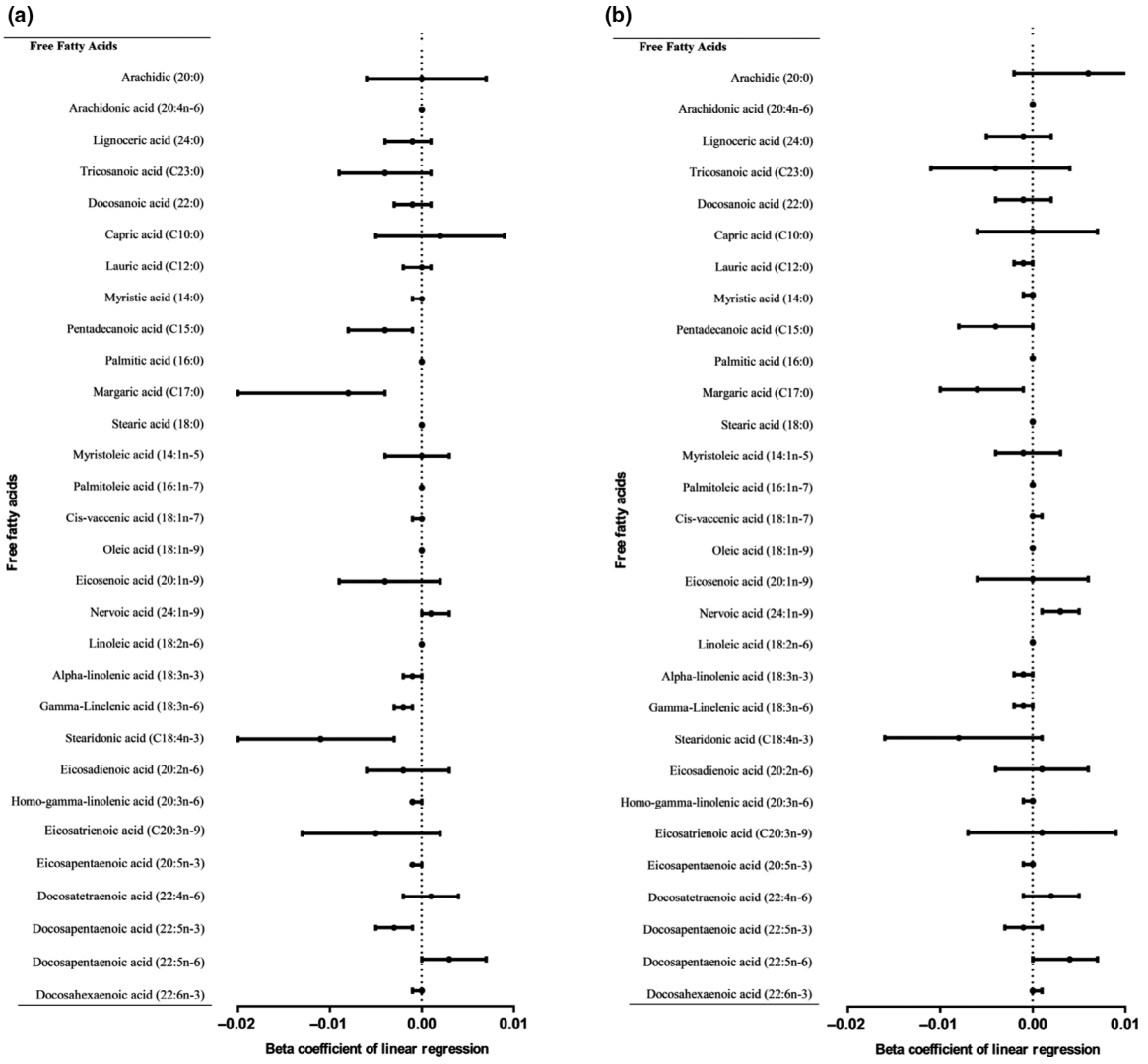

Figure 2 (a) Association between free fatty acids (FFAs) and testosterone levels for women in an unadjusted model. (b) Association between FFAs and testosterone levels for women in a fully adjusted model. Covariate adjustment for the fully adjusted model: age, race/ethnicity, aspartate aminotransferase, body mass index, serum glucose, creatinine, congestive heart failure, coronary heart disease, angina/angina disease, heart attack.

for the release of luteinising hormone (LH) and its resultant testicular stimulation and testosterone release [27]. Leptin is an adipose-derived hormone that stimulates GnRH neurones. Increased adiposity in obese individuals greatly elevates leptin. Not only does this elevation in leptin cause the hypothalamus to become leptin resistant, but also it directly inhibit Leydig cell testosterone production [27]. Evidence has been obtained showing that lower pulsatile LH secretion, which is often found in obese individuals, is correlated with the suppression of testosterone concentration [29]. The overall picture is consistent with our finding that serum FFAs exhibit a negative correlation with the testosterone level in the study participants.

A study conducted by Mínguez-Alarcón *et al.* [15] identified an inverse relationship of MUFAs with morphologically normal sperm and total testosterone levels among young Spanish men. A negative association was observed between the intake of omega-6 and testicular volume, suggesting that omega-6 could negatively influence testicular function by acting directly on the testis. Conversely, a positive relationship between omega-3 and testicular volume was also discovered in the same study [15]. Although omega-3 might have a positive association with semen volume, a relationship with testosterone was not observed in the analysis for both omega-6 and omega-3. Our results have shown that omega-3 fatty acids, including alpha-linolenic acid, eicosapentaenoic acid and

Table 2 Association between serum free fatty acids and blood levels of testosterone in non-obese subjects

	Non-obese							
	Male participants				Female participants			
	95% CI value				95% CI value			
	Unadjusted	*P*-value	Fully adjusted	*P*-value	Unadjusted	*P*-value	Fully adjusted	*P*-value
Capric acid (C10:0) (μmol L^{-1})	−0.004 (−0.011, 0.004)	0.325	−0.004 (−0.011, 0.003)	0.239	0.005 (−0.004, 0.014)	0.259	0.001 (−0.007, 0.010)	0.761
Myristic acid (14:0) (μmol L^{-1})	−0.001 (−0.001, 0.000)	<0.001	−0.001 (−0.001, 0.000)	<0.001	0.000 (0.000, 0.001)	0.541	0.000 (0.000, 0.001)	0.529
Pentadecanoic acid (C15:0) (μmol L^{-1})	−0.004 (−0.007, −0.001)	0.004	−0.004 (−0.008, −0.001)	0.007	−0.001 (−0.006, 0.003)	0.556	0.000 (−0.004, 0.005)	0.851
Palmitic acid (16:0) (μmol L^{-1})	0.000 (0.000, 0.000)	0.002	0.000 (0.000, 0.000)	0.001	0.000 (0.000, 0.000)	0.616	0.000 (0.000, 0.000)	0.071
Margaric acid (C17:0) (μmol L^{-1})	−0.004 (−0.007, 0.000)	0.027	−0.003 (−0.007, 0.000)	0.063	−0.006 (−0.012, −0.001)	0.014	−0.001 (−0.007, 0.005)	0.737
Stearic acid (18:0) (μmol L^{-1})	0.000 (0.000, 0.000)	0.024	0.000 (0.000, 0.000)	0.005	0.000 (0.000, 0.000)	0.248	0.000 (0.000, 0.000)	0.361
Myristoleic acid (14:1n-5) (μmol L^{-1})	−0.005 (−0.008, −0.001)	0.007	−0.005 (−0.009, −0.002)	0.004	0.002 (−0.002, 0.006)	0.302	0.002 (−0.003, 0.006)	0.465
Oleic acid (18:1n-9) (μmol L^{-1})	0.000 (0.000, 0.000)	0.003	0.000 (0.000, 0.000)	0.005	0.000 (0.000, 0.000)	0.298	0.000 (0.000, 0.000)	0.713
Nervonic acid (24:1n-9) (μmol L^{-1})	0.001 (−0.001, −0.003)	0.518	0.001 (−0.001, 0.004)	0.284	0.000 (−0.002, 0.002)	0.934	0.003 (0.001, 0.005)	0.012
Linoleic acid (18:2n-6) (μmol L^{-1})	0.000 (0.000, 0.000)	0.675	0.000 (0.000, 0.000)	0.339	0.000 (0.000, 0.000)	0.061	0.000 (0.000, 0.000)	0.206
Alpha-Linolenic acid (18:3n-3) (μmol L^{-1})	−0.001 (−0.002, −0.001)	<0.001	−0.001 (−0.002, −0.001)	<0.001	−0.001 (−0.002, 0.000)	0.011	−0.001 (−0.001, 0.000)	0.181
Eicosapentaenoic acid (20:5n-3) (μmol L^{-1})	−0.001 (−0.001, 0.000)	0.046	0.000 (−0.001, 0.000)	0.157	−0.001 (−0.001, 0.000)	0.018	0.000 (−0.001, 0.001)	0.953
Docosapentaenoic acid (22:5n-6) (μmol L^{-1})	−0.004 (−0.008, 0.001)	0.138	−0.004 (−0.009, 0.001)	0.133	0.006 (0.001, 0.010)	0.008	0.006 (0.001, 0.010)	0.011
Docosahexaenoic acid (22:6n-3) (μmol L^{-1})	0.000 (−0.001, 0.000)	0.034	0.000 (−0.001, 0.000)	0.232	0.000 (−0.001, 0.000)	0.644	0.001 (0.000, 0.001)	0.011
Docosanoic acid (22:0) (μmol L^{-1})	−0.002 (−0.005, 0.000)	0.080	−0.004 (−0.007, 0.000)	0.031	−0.003 (−0.006, −0.001)	0.017	−0.002 (−0.006, 0.002)	0.314
Tricosanoic acid (C23:0) (μmol L^{-1})	−0.003 (−0.009, 0.004)	0.414	−0.003 (−0.012, 0.005)	0.417	−0.008 (−0.014, −0.002)	0.013	−0.004 (−0.013, 0.005)	0.358
Eicosadienoic acid (20:2*n*-6) (μmol L^{-1})	−0.002 (−0.007, 0.002)	0.255	−0.003 (−0.008, 0.001)	0.158	0.000 (−0.005, 0.005)	0.951	0.003 (−0.003, 0.008)	0.311
Eicosenoic acid (20:1*n*-9) (μmol L^{-1})	−0.003 (−0.007, 0.001)	0.159	−0.002 (−0.006, 0.002)	0.361	0.000 (−0.006, 0.006)	0.931	0.003 (−0.003, 0.010)	0.310

Covariate adjustment for fully adjusted model: age, race/ethnicity, aspartate aminotransferase, body mass index, serum glucose, creatinine, congestive heart failure, coronary heart disease, angina/angina disease and heart attack. CI, confidence Interval.

Table 3 Association between serum free fatty acids and blood levels of testosterone in obese subjects

	Obese							
	Male participants				Female participants			
	95% CI value				95% CI value			
	Unadjusted	*P*-value	Fully adjusted	*P*-value	Unadjusted	*P*-value	Fully adjusted	*P*-value
Capric acid (C10:0) (μmol L^{-1})	−0.012 (−0.022, −0.003)	0.012	−0.014 (−0.023, −0.004)	0.005	−0.003 (−0.015, 0.009)	0.652	−0.004 (−0.015, 0.008)	0.558
Myristic acid (14:0) (μmol L^{-1})	−0.001 (−0.001, 0.000)	0.010	−0.001 (−0.001, 0.000)	<0.001	−0.001 (−0.002, 0.000)	<0.001	−0.001 (−0.002, 0.000)	0.001
Pentadecanoic acid (C15:0) (μmol L^{-1})	−0.007 (−0.012, −0.002)	0.003	−0.013 (−0.018, −0.008)	<0.001	−0.009 (−0.014, −0.004)	<0.001	−0.009 (−0.014, −0.004)	<0.001
Palmitic acid (16:0) (μmol L^{-1})	0.000 (0.000, 0.000)	0.045	0.000(0.000, 0.000)	<0.001	0.000 (0.000, 0.000)	0.002	0.000(0.000, 0.000)	0.011
Margaric acid (C17:0) (μmol L^{-1})	−0.007 (−0.012, −0.001)	0.021	−0.011 (−0.017, −0.005)	<0.001	−0.012 (−0.017, −0.006)	<0.001	−0.011 (−0.017, −0.005)	<0.001
Stearic acid (18:0) (μmol L^{-1})	0.000 (0.000, 0.000)	0.359	0.000 (−0.001, 0.000)	0.004	0.000 (−0.001, 0.000)	0.006	0.000 (−0.001, 0.000)	0.014
Myristoleic acid (14:1*n*-5) (μmol L^{-1})	−0.003 (−0.009, 0.003)	0.309	−0.006 (−0.012, 0.000)	0.043	−0.010 (−0.017, −0.003)	0.005	−0.009 (−0.016, −0.002)	0.008
Oleic acid (18:1n-9) (μmol L^{-1})	0.000 (0.000, 0.000)	0.061	0.000 (0.000, 0.000)	0.001	0.000 (0.000, 0.000)	<0.001	0.000 (0.000, 0.000)	0.007
Nervonic acid (24:1*n*-9) (μmol L^{-1})	0.002 (−0.001, 0.006)	0.211	−0.001 (−0.005, 0.003)	0.639	0.003 (0.000, 0.005)	0.041	0.004 (0.001, 0.007)	0.024
Linoleic acid (18:2n-6) (μmol L^{-1})	0.000 (0.00, 0.000)	0.516	0.000 (0.000, 0.000)	0.018	0.000 (0.000, 0.000)	0.119	0.000 (0.000, 0.000)	0.159
Alpha-linolenic acid (18:3*n*-3) (μmol L^{-1})	−0.001 (−0.002, −0.000)	0.036	−0.001 (−0.002, 0.000)	0.004	−0.002 (−0.003, −0.001)	0.003	−0.001 (−0.003, 0.000)	0.019
Eicosapentaenoic acid (20:5*n*-3) (μmol L^{-1})	−0.001 (−0.003, 0.000)	0.067	−0.002 (−0.003, 0.000)	0.017	−0.001 (−0.002, 0.000)	0.038	−0.001 (−0.002, 0.000)	0.121
Docosapentaenoic acid (22:5*n*-6) (μmol L^{-1})	−0.001 (−0.009, 0.006)	0.761	−0.006 (−0.013, 0.002)	0.141	−0.005 (−0.010, 0.001)	0.121	−0.004 (−0.010, 0.002)	0.206
Docosahexaenoic acid (22:6*n*-3) (μmol L^{-1})	−0.001 (−0.002, 0.000)	0.023	−0.001 (−0.002, 0.000)	0.011	0.000 (−0.001, 0.000)	0.494	0.000 (−0.001, 0.001)	0.735
Docosanoic acid (22:0) (μmol L^{-1})	0.006 (0.002, 0.011)	0.002	0.004 (−0.003, 0.010)	0.255	0.001 (−0.002, 0.005)	0.430	0.001 (−0.004, 0.005)	0.718
Tricosanoic acid (C23:0) (μmol L^{-1})	0.009 (−0.001, 0.019)	0.089	−0.008 (−0.023, 0.008)	0.317	−0.002 (−0.01, 0.006)	0.703	−0.004 (−0.015, 0.008)	0.546
Eicosadienoic acid (20:2*n*-6) (μmol L^{-1})	0.001 (−0.005, 0.007)	0.739	−0.007 (−0.013, 0.000)	0.048	−0.006 (−0.014, 0.002)	0.128	−0.002 (−0.011, 0.007)	0.611
Eicosenoic acid (20:1*n*-9) (μmol L^{-1})	−0.001 (−0.008, 0.006)	0.799	−0.005 (−0.013, 0.002)	0.145	−0.014 (−0.024, −0.003)	0.009	−0.006 (−0.017, 0.006)	0.328

Covariate adjustment for fully adjusted model: age, race/ethnicity, aspartate aminotransferase, body mass index, serum glucose, creatinine, congestive heart failure, coronary heart disease, angina/angina disease and heart attack. CI, confidence Interval.

docosahexaenoic acid, all display a significant negative association with the testosterone level of men in the fully adjusted model ($P < 0.05$). The mechanism behind this association, however, still requires further research.

Recent studies have illustrated the inverse relationship between dietary fat intake and measurements such as testosterone level and total sperm count [15,30-33]. However, it has also been proposed that ingested fats are weakly associated with serum FFAs [34]. Zhang *et al.* [35] discovered a significant relationship between plasma *trans*-fatty acids and metabolic syndrome. It was claimed that total saturated fatty acid and PUFA in the form of serum measurement may not reflect an individual's usual dietary intake [35]. Insulin plays a critical role in the regulation of free fatty acids. Insulin suppresses lipolysis at the same time as maintaining a constant rate of intraadipocyte FFA re-esterification [36]. Such regulation is dependent on factors such as hormones and physical exercise. Hormones such as 17β-oestradiol depolarise β cells through the influx of extracellular calcium ions, causing subsequent insulin secretion [37]. Physical exercise reduces the systemic insulin concentration and improves hepatic and adipose tissue insulin sensitivity [38]. Thus, the concentration of liberated serum FFAs from lipolysis will not correlate with the level of ingested fats in the presence of insulin. We hypothesise that serum FFAs could have a physiological influence on steroid-producing organs otherwise observed from dietary fats. Among the serum FFAs examined in the present study, oleic acid not only reduced testosterone production, but also inhibited the hydrolysis of cholesteryl esters, which was essential for steroidogenesis in the gonads and adrenal cortices [39]. Serum FFAs have a substantial effect on testosterone levels by interfering with enzyme activity and transport. However, the mechanism regarding our claim remains unclear. Further research is needed to confirm this hypothesis.

Furthermore, our age-based analysis revealed a significant inverse relationship between serum FFAs and testosterone levels across all ages. Although it is evident that lower testosterone levels are generally found in older men compared to younger men [40,41], our finding implies the same relationship in other age groups prior to the age of 60 years. This implies that the incorporation of serum FFAs in our study may be the key contributing factor in the reduction in testosterone levels in the non-elderly groups. Such a trend was also present in the women, although it was much less evident.

The present study explored the associations of both saturated and unsaturated fatty acids with testosterone. Increased saturated fat intake was associated with a reduced sperm count and concentration [14]. A similar trend was observed in unsaturated fat, where both MUFAs and PUFAs showed reverse associations with post-prandial testosterone over a 5-h period [42]. Despite having similar trends in testosterone levels, saturated and unsaturated fat imposed distinguishing effects on other aspects. In a systematic review and regression analysis, Mensink [2] discovered a significant increase in total cholesterol for every 1% of dietary energy as *cis*-PUFA replaced with an equal amount of saturated fatty acid. Conversely, replacement with unsaturated fatty acids did not elevate cholesterol levels. Saturated fat also induces the production of inflammatory cytokines and interferons by binding to Toll-like receptor 4 [43]. This effect, however, was not observed for unsaturated fatty acids [43].

The present study has some limitations. The NHANES had a cross-sectional design; thus, causal relationships between serum FFAs and blood levels of testosterone could not be drawn. A long-term observation period should be considered in future studies. Among the analyses presented in our study, we lacked an evaluation of dietary intake. The absence of dietary intake evaluation hindered us from making direct comparisons and associations with serum free fatty acids. Further analysis involving dietary intake could be performed in future studies. In addition, information about the subjects' medical histories was solely based on self-reported responses to questionnaires. Thus, the effect of recall bias and several other errors could not be excluded.

Conclusions

The present study has highlighted the role of serum FFAs in the reduction of testosterone levels. We analysed the relationship in both men and women and found similar trends throughout the study participants. We discovered a negative association between serum FFAs and blood levels of testosterone and postulated a potential mechanism behind the association. Most importantly, serum FFAs may impose an unknown physiological effect on our body that cannot be predicted based on the ingested fats. As a result of the existing uncertainty, further speculation and research, specifically on serum FFAs, are required to verify our conclusion.

Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.
No funding declared.
C-CK and W-LC contributed to the design of the study, were responsible for the management and retrieval of data, contributed to initial data analysis and interpretation, and drafted the initial manuscript. C-CK, Z-YY, Y-WC and W-LC decided upon the data collection methods. C-CK, Z-YY, Y-WC and W-LC were also responsible for the data analysis decisions. W-LC conceptualised

and designed the study, supervised all aspects of the study, critically reviewed and revised the manuscript. All authors read and approved the final manuscript submitted for publication.

Transparency statement

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Association between serum free fatty acids and blood levels of testosterone in subjects aged under 60 years.

Table S2. Association between serum free fatty acids and blood level of testosterones in subjects aged 60 years or over.

ANTHOPOMETRY

How does muscularity assessed by bedside methods compare to computed tomography muscle area at intensive care unit admission? A pilot prospective cross-sectional study

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Abstract

Background: Low muscularity and malnutrition at intensive care unit (ICU) admission have been associated with negative clinical outcomes. There are limited data available evaluating the validity of bedside techniques to measure muscle mass in critically ill adults. We aimed to compare bedside methods for muscle mass assessment [bioimpedance spectroscopy (BIS), arm anthropometry and subjective physical assessment] against reference technology [computed tomography (CT)] at ICU admission.
Methods: Adults who had CT scanning at the third lumbar area <72 h after ICU admission were prospectively recruited. Bedside methods were performed within 48 h of the CT scan. Pearson's correlation compared CT muscle area with BIS-derived fat-free mass (FFM) (kg) and FFM-Chamney (kg) (adjusted for overhydration), mid-upper arm circumference (cm) and mid-arm muscle circumference (cm). Depleted muscle stores were determined using published thresholds for each method. Cohen's kappa (κ) was used to evaluate the agreement between bedside and CT assessment of muscularity status (normal or low).
Results: Fifty participants were enrolled. There were strong correlations between CT muscle area and FFM values and mid-arm muscle circumference ($P < 0.001$). Using FFM-Chamney, all six (100%) participants with low CT muscle area were detected ($\kappa = 0.723$). FFM-BIS, arm anthropometry and subjective physical assessment methods detected 28%–38% of participants with low CT muscle area.
Conclusions: BIS-derived FFM using an adjustment algorithm for overhydration was correlated with CT muscle area and had good agreement with muscularity status assessed by CT image analysis. Arm anthropometry and subjective physical assessment techniques were not able to reliably detect participants with low CT muscle area.

Introduction

Malnutrition at intensive care unit (ICU) admission has been associated with increased ICU length of stay, ICU readmission and mortality.[1] International clinical guidelines for nutrition in the critically ill recommend early nutrition therapy for malnourished patients who are admitted to the ICU.[2,3] Reduced muscle mass is highly related with malnutrition and the Global Leadership on Malnutrition (GLIM) consensus group has included reduced muscle mass in the recently published criteria for diagnosing malnutrition.[4] Furthermore, low muscularity on admission to the ICU has independently been associated with mortality and increased length of stay and may be an important predictor of outcome.[5,6] Despite the developing evidence base and guideline recommendations highlighting the importance of identifying patients with lower than normal muscularity,[4,7] few studies have evaluated how bedside body composition methods perform compared to reference technology in the ICU setting with respect to identifying this phenotype.[8]

The paucity of studies evaluating the accuracy of bedside methods to assess muscularity is primarily a result of logistical and practical challenges. Transporting critically ill patients out of the ICU for body composition assessment using a reference technology (e.g. dual-energy X-ray absorptiometry) is neither feasible, nor a clinical priority. However, in recent years, computed tomography (CT) image analysis at the third lumbar (L3) area, using scans performed for clinical diagnostic purposes has evolved as a body composition method, with skeletal muscle area being highly related to whole-body muscle.[9-11] The method is reliable and precise and is considered as a reference body composition technique for defining sarcopenia in cancer and other populations.[9] As a result, it has become possible to evaluate how bedside assessment of muscularity compares to CT-measured muscularity in critically ill patients who have had a CT scan performed for clinical purposes. CT image analysis also allows for the measurement of muscle density (a marker for muscle quality) at the L3 area, which enables the evaluation of how bedside measures relate to not only muscle mass, but also muscle quality.

The bedside tools for assessment of muscularity recommended by GLIM in general hospital populations, and supported by the European Society of Parenteral and Enteral Nutrition (ESPEN) ICU clinical guidelines, include: bioimpedance technology; subjective physical assessment of muscle stores (using a published tool); and arm anthropometry (mid-upper arm circumference, mid-arm muscle circumference).[3,4] Bioimpedance is based on the measurement of the opposition (impedance) to an electrical current by body tissues. Available technologies include single- and multi-frequency bioelectrical impedance analysis (BIA) and bioelectrical impedance spectroscopy (BIS). Variables relevant for muscularity assessment include: 50-kHz phase angle and estimates of fat-free mass (FFM) (kg).[12] Phase angle, which is generated from the arctangent of the ratio of reactance to resistance at 50 kHz, may be related to cellular health and nutrition status and has been independently associated with ICU mortality and length of stay on ICU admission.[13,14] The estimation of FFM requires the use of population-specific predictive equations, which are based on various assumptions (e.g. normal hydration of lean tissue and fluid distribution), and these are often violated in critical illness as a result of large fluid shifts and oedema.[15,16] A conceptual model (Chamney model) has been developed from cadaver data and applied to BIS data in dialysis populations, and involves an adjustment for excess fluid/overhydration based on normal hydration of lean and adipose tissue.[17] The model has been applied to fluid management in dialysis patients but has not yet been investigated in the ICU setting.[18] Subjective physical assessment and arm anthropometry techniques for muscle mass assessment may also be influenced by fluid status, and further evaluation against a reference method is required to understand the utility of the methods to assess muscularity and accurately detect muscle depletion.

The aims of this pilot study were to: (i) determine the association between bedside measures of muscle mass [BIS-derived FFM (unadjusted and fluid adjusted) and arm anthropometry] and a reference method (CT muscle area) at ICU admission; (ii) evaluate how BIS-derived phase angle relates to CT muscle area and density; and (iii) assess the agreement between muscularity status (low or normal) assessed by BIS, arm anthropometry and subjective physical evaluation of muscle stores and CT image analysis.

Materials and methods

Study design and setting

This prospective cross-sectional observational study was conducted in a single centre ICU between January 2017 and March 2019 after approval from the Research and Ethics Committees at The Alfred hospital and La Trobe University. The data presented were collected as part of a larger study.[19] The study was registered *a priori* on clinicaltrials.gov (NCT03019913). Written and informed consent were obtained from eligible patients and/or their legal medical decision-maker. Reporting of the study follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[20]

Participant selection

Patients were eligible if they were aged ≥18 years and had a CT scan including the L3 area performed for clinical reasons ≤24 h *before* or ≤72 h *after* ICU admission. Exclusion criteria were: the CT scan was unanalysable for muscle assessment at the L3 area, death was imminent, anticipated ICU stay was <24 h, pregnancy, body mass index (BMI) >40 kg/m^2, it was impractical and/or not possible to complete bedside measurements or it was not possible to obtain informed consent. To limit the time between the CT scan and bedside methods, patients were also excluded if the CT scan was performed ≥48 h prior to enrolment. Baseline demographic and clinical data, including age, sex, Charlson Comorbidity Index, Acute Physiologic and Chronic Health Evaluation (APACHE) II and III score, admission diagnosis (trauma, medical or surgical), ICU and hospital length of stay, were collected for all participants [21-23]. Weight (kg) recorded was the pre-admission (dry) weight obtained from the patient or family or estimated by an experienced dietitian (KJL) by visual assessment (taking into account any apparent fluid overload). Height (m) was either reported by the family or estimated. For descriptive purposes, BMI (kgm^{-2}) was calculated. BMI category was determined using the World Health Organization (BMI) cut-off values (underweight <18.5 kg m^{-2}, normal weight 18.5–24.9 kgm^{-2}, overweight 25–29.9 kg m^{-2}, obese >30 kg m^{-2}).[24] Fluid balance in the 24-h period before performing the bedside protocol was recorded (where 24 h of data was documented in the medical record).

Computed tomography image analysis

During the screening process for eligibility, investigators visualised skeletal muscle area at L3 and, where necessary, an experienced radiologist (GSG) confirmed the quality of the scan was adequate for analysis. Patients were excluded if the muscle borders were indistinguishable, if there was interference of artifact or if whole muscle group(s) were not visible as a result of positioning during CT scanning. CT scans were uploaded onto the licensed software, SLICEOMATIC, version 5.0 (TomoVision, Montreal, QC, Canada) for analysis. A trained investigator (KJL) identified the slice for analysis at L3, and skeletal muscle boundaries were recognised based on Hounsfield units (–29 to +150 for muscle).[25] Abdominal muscle cross-sectional area (cm^2) was automatically computed by the software by summing the skeletal muscle tissue pixels and multiplying by the surface area of each pixel. Skeletal muscle density (Hounsfield units) was also automatically computed by the software by calculating the mean radiological muscle attenuation of all muscle visible at the L3 level.

Bioimpedance spectroscopy

BIS was performed within 48 h of the CT scan using the ImpediMed SFB7 BIS device (ImpediMed Limited, Pinkenba, QLD, Australia). The BIS device scans 256 frequencies between 3 and 1000 kHz and using Cole modelling and equations incorporating Hanai mixture theory, the software determines total body water (TBW), extracellular water (ECW) and intracellular water (ICW).[12] The BIS measurement was performed when the patient was supine with the head of the bed at approximately 30-degrees (usual positioning in our ICU) and with the limbs separated.[12] Participants had been bed-bound for >12 h prior to measurement. The dorsal surface of the hands and feet were cleaned with alcohol and device-specific electrodes were placed 5 cm apart: two on the hands and two on the feet. The leads were attached to the electrodes and the measurement recorded (at 10-s intervals for 1 min). The leads were then removed. At least 5 min later, with the patient in the same position, the leads were reconnected and a second measurement was taken. The data were then uploaded into the Impedimed software program (BIOIMP, version 5.5.0.1) and modelled results, including raw data, and ECW, ICW, TBW and FFM (kg), were exported into EXCEL (Microsoft Corp., Redmoind, WA, USA) for further interpretation. The mean values from the two measurements were used for analysis.

FFM (kg) was estimated from TBW measures by the Impedimed SFB7 software and was recorded as *FFM-BIS*. A modified FFM (kg) variable was also calculated using the Chamney model (equation detailed below for the Chamney 'normally hydrated lean tissue' variable), which accounts for excess fluid and is relabeled here for ease of comparisons as *FFM-Chamney* [17]:

FFM-Chamney = (2.725 × ICW) + (0.191 × Chamney Excess Fluid) – (0.191 × weight). With Chamney Excess Fluid = (1.136 × ECW) – (0.430 × ICW) – (0.114 × weight).

Phase angle at 50 kHz was also recorded.

To avoid including FFM values that were not reflective of muscle mass status (e.g. extreme fluid overload) a measurement was accepted and used for analysis if it met the following criteria: Cole plot followed a half semi-circular pattern, standard error of estimation (SEE) for fit to the curve below 1.0, intracellular resistivity (Ri) *greater* than extracellular resistivity (Re) and whole body FFM within physiological limits (e.g. none of the water or FFM values larger than body weight).[26] The software fitted the resistance and reactance spectral data to a semi-

circular Cole model, from which key model terms were derived and applied to the software algorithm using the default analysis parameters, which included data from 10 to 500 kHz, and automatic correction for time delay (i.e. high frequency capacitance). Rejection limits (up to 10%) were applied in an attempt to exclude outliers when SEE were >1.0, and data were included if all the criteria were met after these limits were applied.

Arm anthropometry

Mid-upper arm circumference (cm) was measured at the mid-point between the tip of the acromion and the olecranon process.[27] Triceps skinfold thickness (mm) was measured using Harpenden skinfold calipers (John Bull, British Indicators Ltd, Weybridge, UK), which were applied to the posterior surface of the fully relaxed and lifted arm, at the same marked point.[27] Measurement was recorded to the nearest millimetre and converted to centimetres for analysis. Mid-arm muscle circumference was calculated using mid-upper arm circumference and triceps skinfold thickness, using the formula:

Mid-arm muscle circumference (cm) = mid-upper arm circumference (cm) – [3.142 × tricep skinfold thickness (cm)]

Where possible, measurements were taken on the right side (or left if right was not available). Two measurements were taken at each point and the average used for analysis.

Physical assessment of muscle wasting

A trained investigator (KJL) undertook a subjective assessment of muscle wasting (none, mild–moderate or severe) using the physical assessment section of the widely used subjective global assessment (SGA) tool.[28]

Assessment of muscularity status

Muscularity status (normal or low) was determined using published thresholds for each of the methods. Low CT muscle area (reference method) was classified using cut-points derived from a general ICU population where low CT muscle area was associated with increased mortality (<170 cm^2 males and <110 cm^2 females).[6] For BIS, international guideline thresholds for low FFM index (FFM divided by height in metres squared) were used (<17 kg m^{-2} males and <15 kg m^{-2} females) [4,29]. Low muscularity using arm anthropometry measures was determined as <15th percentile using the age and sex-specific data from the 2007–2010 National Health and Nutrition Examination Survey[31], which was the most recent data set with tricep skin fold measurements. This value was chosen, based on a previous study which reported that mid-upper arm circumference <15th percentile predicted mortality in a group of critically ill patients.[27] For subjective physical assessment, low muscularity was recorded for participants who displayed mild-moderate or severe muscle wasting using the SGA tool.

Statistical analysis

For this pilot study, a pragmatic sample size of 50 patients was chosen. SPSS, version 25 (IBP Corp., Armonk, NY, USA) was used for all analyses. Shapiro–Wilk tests were used to assess normality. Data are reported as *n* (%), mean (SD), or median and interquartile range (IQR). Missing data were not imputed. Differences in mean CT muscle area and bedside methods of assessing muscularity by sex and age (<65 years versus ≥65 years) were assessed using independent Student's *t* tests.[30] Pearson's correlation was used to assess the relationship between CT muscle area and bedside measures.

Cohen's kappa statistic (κ) was used to evaluate the agreement between muscularity status (normal or low) assessed by the bedside methods and CT image analysis. For all analyses, $P < 0.05$ was considered statistically significant.

Results

Participants

Of 1580 patients were screened, and of the 373 patients who had a CT scan including the L3 area, 323 patients were excluded, leaving 50 participants included in the primary study.[19] Of these, all participants had a subjective physical assessment, 41 (82%) had arm anthropometry and 26 (52%) had BIS data recorded. The CONSORT diagram is shown in Figure 1.

Participants were predominantly Caucasian, male [38 (76%)], young [<65 years old (33 (66%)] and admitted post-trauma injury [42 (84%)]. Participant characteristics for the entire cohort of 50 patients and for the subgroups with valid BIS and arm anthropometry measurements are shown in Table 1. The mean (SD) time from ICU admission to performing the bedside measurements was 33 (12) h, and that from CT scan to the bedside measurements was 26 (13) h. Mean values for CT muscle area, FFM, phase angle, mid-upper arm circumference, mid-arm muscle circumference; by age and sex are shown in Table 2. Mean (SD) fluid balance for the 24-h period before performing the bedside protocol was +1726 (1354) mL for the total cohort ($n = 31/50$), +1755 (1266) mL for the group with BIS measurements

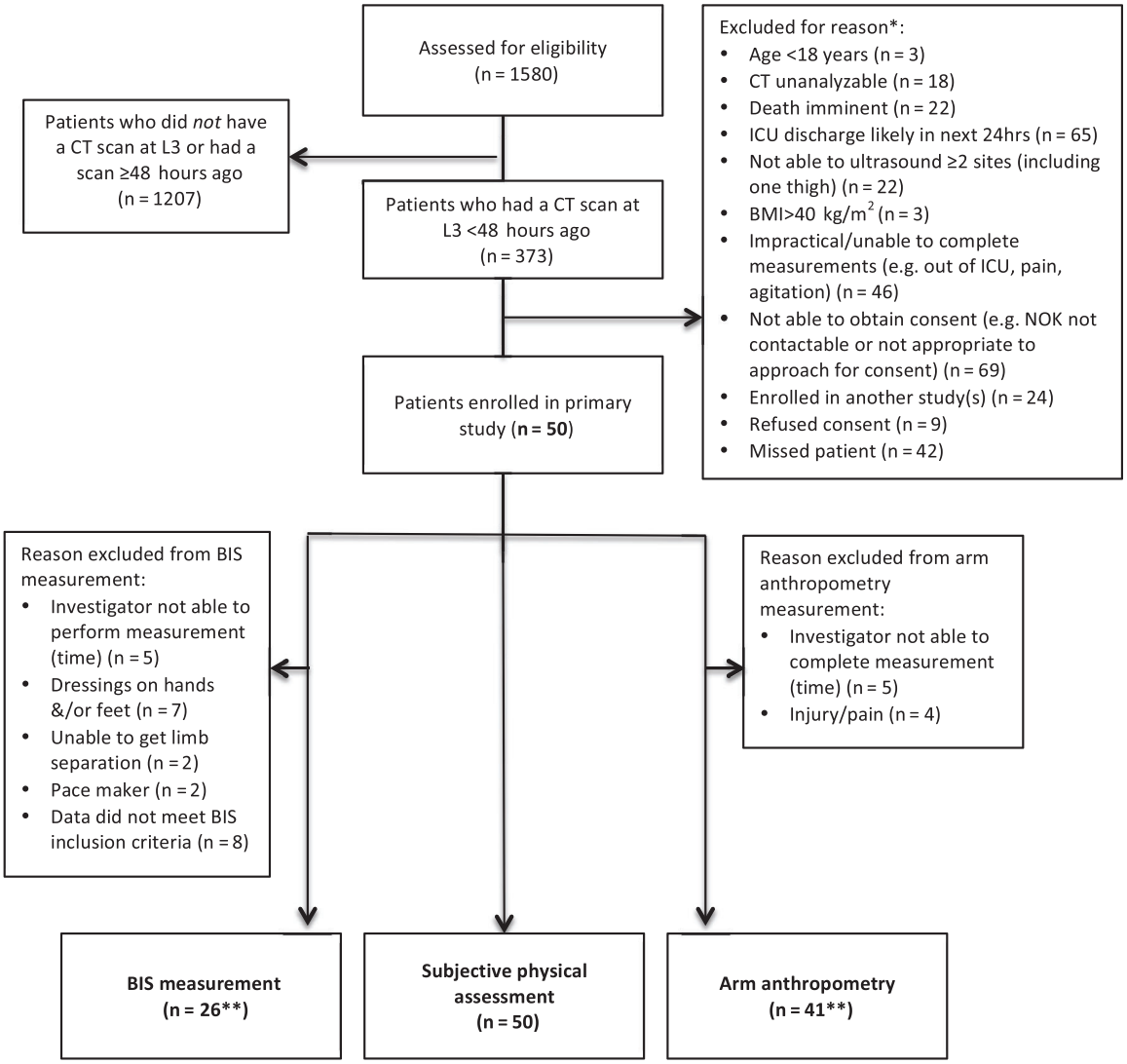

Figure 1 CONSORT diagram. BMI, body mass index; BIS, bioimpedance spectroscopy; CT, computed tomography; L3, third lumbar; NOK, next of kin. *Reasons for exclusion were based on primary study [19]. **Number of patients out of the 50 patients enrolled in the primary study [19] who had a valid measurement.

(n = 18/26) and +1748 (1248) mL for the group with arm anthropometry measurements (n = 26/41).

Correlation between fat-free mass and arm anthropometry and computed tomography muscle area

There were strong positive and significant correlations between CT muscle area and FFM-BIS (kg) (r = 0.801, P < 0.001) and FFM-Chamney (kg) (r = 0.807, P < 0.001), Figure 2. Mid-arm muscle circumference was more strongly correlated with CT muscle area than mid-upper arm circumference (r = 0.665, P < 0.001 versus r = 0.342, P = 0.029) (Figure 2).

Correlation between phase angle and computed tomography muscle area and density

Phase angle was significantly correlated with CT muscle area (r = 0.589, P < 0.001) and CT muscle density (r = 0.776, P < 0.001) (Figure 3).

Agreement between bedside and computed tomography assessment of muscularity status

In the group who had BIS measurements (n = 26), there were six (23%) participants who had low CT muscle area. Using FFM-BIS values, two (33%) participants were

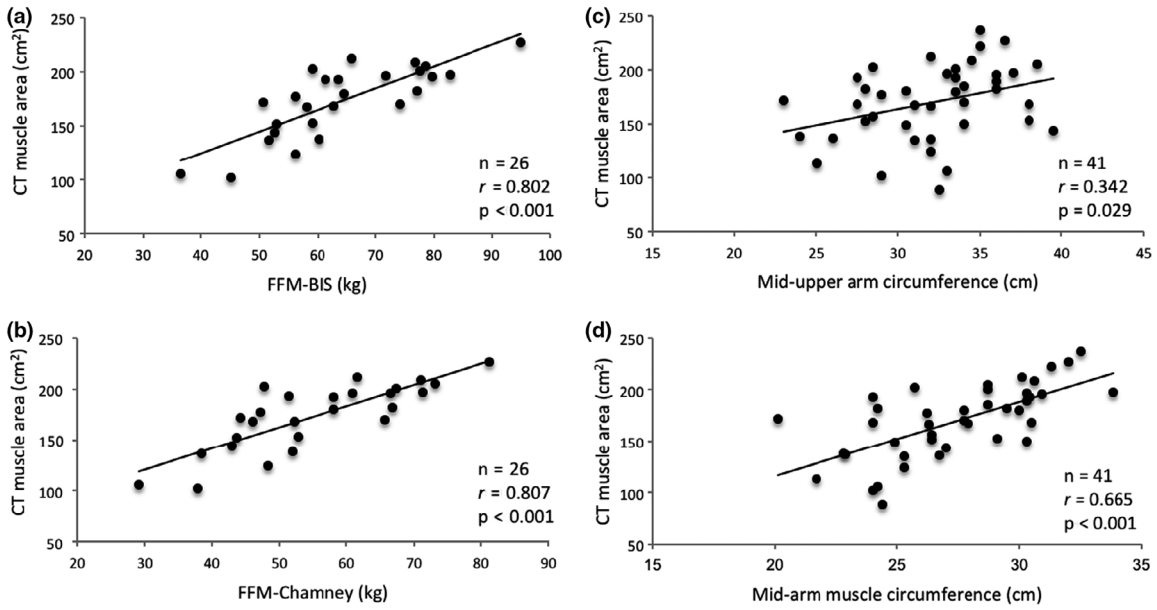

Figure 2 Correlation between computed tomography (CT) muscle area, fat-free mass (FFM)- Bioimpedance spectroscopy (BIS) (a), FFM-Chamney (b), mid-upper arm circumference (c), and mid-arm muscle circumference (d).

correctly classified as having low CT muscle area [$\kappa = 0.435$, 95% confidence interval (CI) = 0.016–0.854, $P = 0.007$]. Using FFM-Chamney values, all six (100%) participants who had low CT muscle area were detected ($\kappa = 0.723$, 95% CI = 0.441–1.00, $P < 0.0005$).

In the 41 participants who had arm anthropometry measurements, 13 (32%) had low CT muscle area. Anthropometry had a poor ability to classify participants with low CT muscle area, with four (31%) being correctly classified using mid-upper arm circumference ($\kappa = 0.060$, 95% CI = −0.249–0.309, $P = 0.698$) and five (38%) using mid arm muscle circumference ($\kappa = 0.137$, 95% CI = −0.178 to 0.452, $P = 0.378$).

In the total cohort ($n = 50$), 14 (28%) participants had low CT muscle area. Of these, four (28%) participants were correctly classified as having low CT muscle area using the physical assessment tool ($\kappa = 0.365$, 95% CI = 0.093–0.637 $P = 0.001$).

Discussion

In this exploratory prospective observational study, we report that BIS-derived FFM, adjusted using the Chamney model, which accounts for fluid overload, was significantly correlated with CT muscle area and had good agreement with muscularity status assessed by CT image analysis. Other bedside methods; FFM-BIS (i.e. unadjusted), arm anthropometry and subjective physical assessment, although correlated with CT muscle area, performed poorly in correctly classifying participant muscularity status. BIS-derived phase angle, which has been identified as a potential predictor of outcome in critically ill, had a stronger relationship to CT muscle *density* than to CT muscle area.

Recently, two prospective single-centre studies investigated how muscle mass derived from bioimpedance techniques relates to CT muscle area in critically ill adults. In both studies, Looijaard *et al.* [32] (Netherlands) and Kim *et al.* [33] (Korea) compared CT- and BIA-derived indices of muscularity (one using a multifrequency BIA device and the other using a single-frequency BIA device). In agreement with our data, both studies found significant correlations between muscularity assessed by the two methods. However, correlation coefficients do not identify the measurement error (agreement) between two techniques.[12] To do this, variables must be converted using prediction equations into comparable parameters, which in turn introduces additional assumptions. To assess the agreement between the two methods, the two studies converted CT muscle area (cm^2) into skeletal muscle mass (kg) using the Shen equation [10]. Both studies reported that BIA significantly overestimated SMM; with increasing disagreement at higher muscle mass. Kim *et al.* [33] reported a significant mean (SD) bias of 3.4 (5.6) kg and wide limits of agreements between CT and MF-BIA-derived muscle mass and Looijaard *et al.* [33] found significant differences between CT and SF-BIA-derived muscle mass values from all three equations used (mean biases 2.4–6.9 kg with wide limits of agreement) [32,33]. Because these types of analyses require the use of multiple muscle

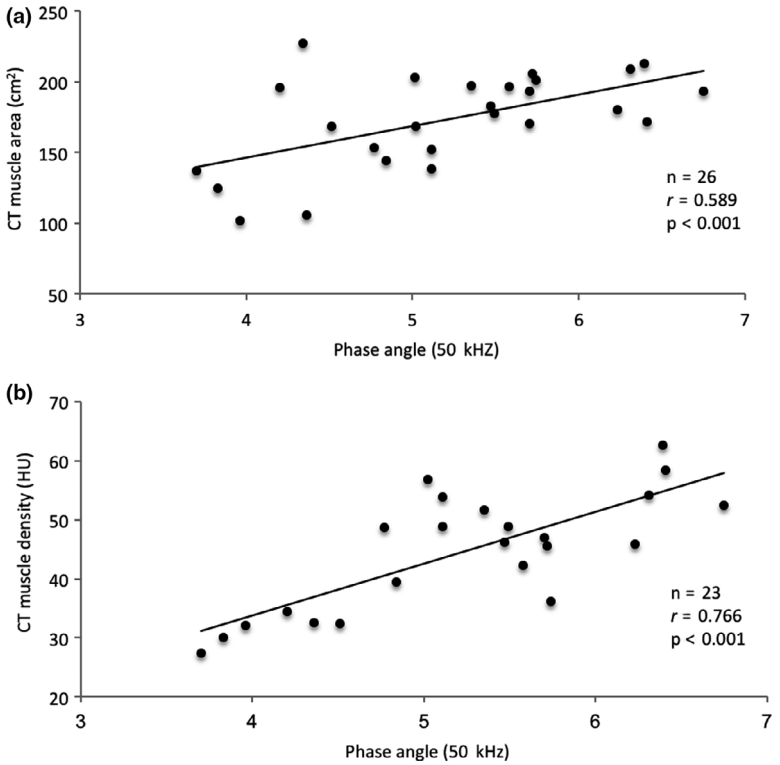

Figure 3 Relationship between phase angle and computed tomography (CT) muscle area (a) and CT muscle density (b).

mass equations, which in turn rely on a range of assumptions that to our knowledge have not been validated for the BIS technology we used, we elected not to undertake such explorations. However, the data from these studies and our study suggest that BIA-derived FFM estimates compared to CT muscle area in the ICU setting are variable and are influenced by hydration status, ethnicity and the equations used to derive FFM values, and so caution should be exercised when interpreting data.

Because unadjusted estimates of FFM in critical illness using bioimpedance technology may be confounded by fluid overload, raw data from bioimpedance devices (which are independent of weight), such as phase angle, are being increasingly explored in the ICU setting. Specifically, phase angle at ICU admission has been associated with increased survival in two prospective observational studies [13,34] and predicted live ICU discharge in another.[35] Although phase angle may be an important predictor of outcome in critically ill patients, the mechanisms for these findings are not entirely understood. Recently, the relationship between phase angle and muscularity has been explored and, in agreement with the findings of the present study, a moderate correlation between phase angle and CT muscle area was reported [32,35]. In the present study, we observed a stronger correlation between phase angle and CT muscle density compared to CT muscle area ($r = 0.776$ and $r = 0.589$, respectively) and these findings are consistent with those of Looijaard *et al.* ($r = 0.701$ versus $r = 0.542$).[32] These data fit with the theory that phase angle is reflective of cell membrane integrity and quality.[36] Further research is required to understand what phase angle threshold is predictive of poor outcome and what changes in phase angle over time correlate with nutritional changes that evolve with the illness course or resulting from nutritional interventions.

Finding a bedside assessment method to identify patients with lower than normal muscularity accurately is a key critical care nutrition research priority.[37] In the present study, the only bedside method that performed well in correctly classifying patients with low CT muscle area was FFM-Chamney values ($\kappa = 0.723$). These findings are in agreement with the study by Looijaard *et al.* [32] mentioned above, who reported that BIA (using the Talluri equation) had a good ability to identify patients with low CT muscle area (area under the curve: males 0.919; females 0.912).[32] These results highlight that bioimpedance technology may be useful to identify patients with low muscularity on ICU admission, although, as we have shown in the present study, accuracy is likely to be dependent on the equation used to derive FFM values and also on whether any adjustment

Table 1 Patient characteristics*

Characteristics	All patients (n = 50)	BIS (n = 26)	Arm anthropometry (n = 41)
Age years, mean (SD)	52 (20)	48 (18)	53 (19)
Age category, *n* (%)			
<65 years	33 (66)	20 (77)	27 (66)
≥65 years	17 (34)	6 (23)	14 (34)
Sex, *n* (%)			
Male	38 (76)	20 (77)	31 (76)
Female	12 (24)	6 (23)	10 (24)
APACHE II	12 (9–16)	12 (9–15)	12 (10–16)
APACHE III	45 (35–65)	43 (33–61)	44 (35–66)
Height m, mean (SD)	1.72 (0.09)	1.71 (0.08)	1.71 (0.08)
Weight kg, mean (SD)	82 (15)	84 (15)	83 (15)
BMI (kg m^{-2}), mean (SD)	28 (5)	28 (5)	28 (5)
Underweight, *n* (%)	1 (2)	0	1 (2)
Normal weight, *n* (%)	15 (30)	6 (23)	10 (24)
Overweight, *n* (%)	18 (36)	12 (46)	16 (39)
Obese, *n* (%)	16 (32)	8 (31)	14 (34)
Comorbidity index, mean (SD)	2 (2)	2 (2)	2 (2)
Admission category, *n* (%)			
Trauma	42 (84)	22 (84)	33 (81)
Medical	7 (14)	3 (12)	7 (17)
Surgical	1 (2)	1 (4)	1 (2)
Patients MV, *n* (%)	31 (62)	17 (65)	26 (63)
ICU LOS, days	5 (2–11)	5 (2–12)	6 (2–11)
Hospital LOS, days	16 (11–24)	15 (8–23)	15 (9–23)

APACHE, Acute Physiology and Chronic Health Evaluation; BMI, body mass index; ICU, intensive care unit; LOS, length of stay; MV, mechanically ventilated.

*Values are presented as the median (interquartile range) unless stated otherwise.

for hydration status is made. It is useful to remember that our fluid-adjusted FFM variable is calculated from ICW and ECW values, and is quite likely to be a more specific representation of the muscle compartment than the more broad FFM variable, which is based on a two-component conceptualisation of body composition. Thus, it is not surprising that this fluid-adjusted FFM variable is more closely aligned with the CT muscle data. Future studies are required to understand how low muscularity assessed by bioimpedance technology relates to clinical and functional outcomes in critically ill adults. Accounting for fluid status may be an important consideration when assessing the ability of bioimpedance technology to predict outcome, as has been shown in renal patients receiving dialysis, with BIS-derived normally hydrated lean tissue (i.e. FFM-Chamney values in the present study) and fluid overload (assessed using the Chamney model) being associated with mortality [38,39].

In the present study, we found that arm anthropometry and subjective physical assessment did not perform well in identifying those patients with low CT muscle area. The accuracy of using mid-upper arm circumference to measure and track changes in muscularity in critically ill patients has been questioned before. In a study by Campbell *et al.* [40], nine patients with multiorgan failure were studied, and muscle thickness (via ultrasound) and mid-upper arm circumference were measured every 1–4 days early in the ICU admission. Using ultrasound, all patients showed a significant, consistent decrease in muscle thickness over time (a finding which has been replicated in subsequent studies).[41] By contrast, the arm circumference measurements showed no consistent pattern of change.[40] It was hypothesised that oedema most likely influenced arm measurements, thus rendering them of low utility.

Similarly, the ability of the SGA tool to identify individuals with low muscularity in critically ill patients has also been challenged. Nutrition assessment using the SGA tool was undertaken in a study of critically ill respiratory patients who had a CT scan at the L3 area, finding that 63% of patients with low CT muscle area were misclassified as normally nourished (where the CT scan and SGA were performed within 3 days of each other).[42] These findings are similar to the present study, where 72% of participants with low CT muscle area were not detected by subjective physical assessment using the SGA tool.

The present study has strengths and limitations. We used standardised methodology to identify and exclude participants with extreme and unphysiological bioimpedance variables (e.g. as a result of fluid overload). These findings contribute to the literature with respect to understanding the capabilities of BIS to provide more specific estimates of muscle, when accounting for patients with extreme fluid overload and/or potential measurement errors (e.g. inadequate limb separation, interference with other bedside machinery). They also importantly highlight that lean tissue depletion may be masked using standard bioimpedance techniques (without adjusting for fluid status). BIS-derived measurements of lean tissue, when adjusted for fluid status using approaches such as Chamney modelling, also show potential for use in clinical practice to use body composition-based approaches for the diagnosis of malnutrition, such as the GLIM. Indeed, this approach could improve on the diagnosis of malnutrition in critical illness and other populations with fluid-overload compared to simpler single-frequency-

Table 2 Values for computed tomography (CT) muscle area and each bedside method by sex and age category*

Variable	*n*	All patients	*n*	Male	*n*	Female	*P* value	*n*	Younger (<65 years)	*n*	Older (≥65 years)	*P* value
(A) CT and BIS												
CT muscle area, cm^2	26	173.4 (33.0)	20	185.9 (23.9)	6	132.9 (26.6)	0.001	20	184.2 (23.1)	6	137.3 (37.1)	0.001
FFM-BIS, kg	26	64.2 (13.2)	20	68.1 (11.9)	6	51.3 (8.8)	0.004	20	68.4 (11.5)	6	50.2 (8.2)	0.001
FFM-Chamney, kg	26	55.2 (12.8)	20	58.6 (11.8)	6	43.8 (9.2)	0.010	20	59.5 (10.8)	6	40.8 (7.2)	0.001
Phase angle, 50 kHz	26	5.3 (0.8)	20	5.5 (0.8)	6	4.5 (0.5)	0.008	20	5.6 (0.7)	6	4.4 (0.6)	0.001
(B) CT and arm anthropometry												
CT muscle area, cm^2	41	170.0 (35.0)	31	182.8 (26.4)	10	130.1 (27.7)	0.001	27	183.1 (28.1)	14	144.7 (28.1)	0.001
Mid-upper arm circumference, cm	41	32 (4)	31	32 (4)	10	32 (4)	0.897	27	33 (5)	14	31 (3)	0.267
Mid-arm muscle circumference, cm	41	27 (3)	31	28 (3)	10	25 (2)	0.009	27	28 (3)	14	26 (2)	0.089

BIS, bioimpedance spectroscopy; CT, computed tomography.
*Values are presented as the mean (standard deviation).

derived FFM cutpoints. Furthermore, the bioimpedance FFM-index cut points provided in the GLIM criteria were derived from a 50-kHz FFM equation developed from Swiss Caucasian population data, and their application to bioimpedance data derived from other devices, as well as in other populations, is inherently limited, and not likely to effectively identify all patients with low muscularity. Despite these limitations, our findings highlight the potential value that a simple BIS-derived fluid-adjusted measure of lean tissue can have with respect to the identification of low muscularity and malnutrition. This is important for identifying those individuals who may need targeted interventions, and also because weight-based indicators, simple anthropometry and subjective physical assessment may not be sensitive enough to detect malnutrition.[42] Limitations of the study include the modest sample size and generalisability of the results, with the majority of participants being young and previously well trauma patients (as a result of a requirement for a CT at L3 to enter the study). Additionally, an estimated weight was used for input into the BIS device (with anticipated challenges with obtaining an accurate weight on ICU admission, e.g. as a result of drain tubes, dressings, etc.). It also remains unclear whether there is a linear relationship between CT muscle area at the L3 region and whole-body muscularity in critically ill patients.

Conclusions

In this pilot study, a unique BIS-derived FFM variable using an equation that accounts for fluid overload was significantly correlated to CT muscle area and was able to correctly classify all the participants with low CT muscle area at ICU admission. Arm anthropometry and subjective physical assessment were not able to readily detect patients with low CT muscle area. Phase angle had a stronger relationship to CT muscle density compared to the muscle area. Future studies should investigate how low muscularity assessed by BIS (ideally using an equation or method to account for fluid overload) relates to clinical and functional outcomes in critically ill patients.

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Conflicts of interest, source of funding and authorship

In the past, CPE has received small monetary support and loaner bioimpedance devices from Bodystat LTD, ImpediMed and InBody. All other authors declare that they have no potential conflicts of interest. KJL was supported by an Australian Government Research Training Scholarship. KJL, CPE, ACT, AF and SJK contributed to the conception and design of the research. KJL, CPE, GSG and SJK contributed to the acquisition and analysis of the data. KJL, CPE and SJK contributed to the interpretation of the data. KJL and SJK drafted the manuscript. All authors critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final version of the manuscript submitted for publication.

Transparency declaration

The lead author affirms that this manuscript is an honest accurate and transparent account of the study being

reported and is compliant with the STROBE guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned (which was approved by the research and ethics committees at The Alfred hospital and La Trobe University) have been explained.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Checklist S1. Checklist of items that should be included in reports of cross-sectional studies.

EARLY LIFE NUTRITION

The relationship between famine exposure in early life and left atrial enlargement in adulthood

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Abstract

Background: Increased left atrium diameter (LAD) is associated with an elevated risk of cardiovascular diseases. The relationship between nutrition status and left atrial enlargement (LAE) is still unclear. The present study aimed to investigate the association of famine exposure in early life with LAE in adulthood.
Methods: Participants were divided into non-exposed, fetal, early, middle and late childhood exposed groups according to birth data. LAE was defined when LAD was ≥3.9 cm in women and ≥4.1 cm in men, or ≥2.3 cm m^{-2} by a sex-independent cut-off normalised for body surface area. Multivariate logistic regression was performed to calculate the odds ratio (OR) and confidence interval (CI) between famine exposure and LAE.
Results: In total, 2522 [905 male, mean (SD) age 59.1 (3.65) years] subjects were enrolled, including 392 (15.5%) LAE subjects. The prevalence of LAE in non-exposed, fetal, early, middle and late childhood exposed groups was 55 (10.8%), 38 (11.2%), 88 (18.1%), 102 (16.7%) and 109 (19.0%), respectively. Compared to the non-exposed group, the ORs for LAE were in fetal (OR = 0.956, 95% CI = 0.605–1.500, $P = 0.847$), late (OR = 1.748, 95% CI = 1.208–2.555, $P = 0.003$), middle (OR = 1.647, 95% CI = 1.140–2.403, $P = 0.008$) and early (OR = 1.630, 95% CI = 1.116–2.399, $P = 0.012$) childhood exposed groups after adjusting potential cofounders. When stratified by gender, smoking, body mass index, hypertension and diabetes, we found that the effect of famine exposure on LAE was only modified by diabetes ($P_{interaction} = 0.007$).
Conclusions: Famine exposure during childhood stage might increase the risk of LAE in adults, and this effect interacts with diabetes.

Introduction

An increased left atrium diameter (LAD), as assessed by echocardiography, is an important manifestation and marker of the changes in cardiac structure and function occurring in response to chronic pressure and volume overload [(1)]. Left atrial enlargement (LAE) is a well-known independent risk factor for cardiovascular and cerebrovascular diseases, such as stroke and atrial fibrillation [(2,3)]. Many factors affect the reconstruction and dilation of the left atrium, and the size of left atrium is regulated by both physiological and pathological factors [(4)]. Numerous epidemiological or cohort studies have shown that LAE is associated with age, obesity, smoking, alcohol consumption, sleep apnoea syndrome, diabetes and hypertension [(5-9)]. Some studies have reported that nutrient status, such as vitamin D deficiency [(10)], triglycerides and fish oil levels [(11)], is related to the left atrial abnormalities. It is generally known that food shortages caused by famine can lead to a large number of nutrient

deficiencies, thus affecting human health or the occurrence of several diseases [12,13]. In recent years, a large number of studies have reported that famine exposure during early life is an independent risk factor for cardiovascular metabolic diseases in adulthood [14,15]. The Dutch Famine of 1944–1945 demonstrated that exposure to famine during gestation and childhood may lead to increase in diabetes, obesity, coronary heart disease, dyslipidaemia, hypertension, abnormal kidney function, mental illness and affective disorders [16,17]. In addition, China has also experienced a severe famine era, with the Chinese famine of 1959–1961 being recognised as one of the severest catastrophic events in Chinese history [18]. Importantly, compared to famines in other countries, such as the Netherlands and Ukraine, the Chinese famine lasted longer, with a major and more extensive impact on the health of the Chinese people [19]. However, currently, there are limited studies on exposure to Chinese famine in early life and LAE in adulthood. Therefore, the present study aimed to investigate the association between exposure to the Chinese famine at different early life stages and the risk of LAE in adulthood.

Materials and methods

Study population

This was a cross-sectional survey study and the data originated from the Early Screening and Comprehensive Intervention Program for High Risk Population of CVD conducted in Guangdong province, China. The Early Screening and Comprehensive Intervention Program for High Risk Population of CVD comprises a population-based national screening study aiming to discover subjects at high risk of cardovascular disease in all 31 provinces (including Guangdong province) in mainland China [20]. There were 10 984 subjects aged 35–75 years who completed the screening survey in Guangdong province between 1 January 2017 and 31 December 2018. Participants completed a cardiac ultrasound examination and LAD data were recorded. However, those participants who did not undergo a cardiac ultrasound examination, as well as those without height or weight measurements or who were suffering heart valve disease and arrhythmia, were excluded. Finally, 2522 subjects were enrolled for the analysis. The research flow chart is presented in Fig. 1.

Data collection

At baseline, standardised questionnaires were obtained face-to-face by trained nurses or community physicians. The content of the questionnaire mainly including sociodemography (such as age, gender, identity number, race, income, education level and marriage), life behaviour and habits (such as smoking, alcohol intake, physical activity and diet), history of chronic diseases (such as hypertension, heart valve disease, arrhythmia and diabetes) and current using of drugs (such as antihypertensive drugs, hypoglycaemic drugs and lipid-lowering drugs). Weight, height, systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate were obtained by standard methods. In addition, fasting blood was used to detect fasting blood glucose (FBG), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C). Body mass index (BMI) was obtained as: weight (kg) divided by the square of height (m^2) [21]. Diabetes was defined as FBG $\geq$126 mg dL^{-1} or via self-reported diagnosis by a healthcare professional or currently using glucose lowering drugs [22]. Hypertension was defined as SBP/DBP $\geq$140/90 mmHg or via self-reported diagnosis by a healthcare professional or as indicated by those currently using antihypertensive drugs [23].

Left atrium diameter measurement

All cardiac ultrasound examinations were performed according to standardised procedures using a Vivid-S6 system (GE Medical System, Milwaukee, WI, USA) interfaced with a 2.5–3.5 MHz phased-array probe. The recordings of cardiac ultrasound examination were evaluated by experienced cardiologists. According to the American Society of Echocardiography guidelines [24], measurements of the left atrium were obtained via the biplane area-length method using the M-Mode two-dimensional technique. The LAD was obtained using a leading edge-to-leading edge measurement of the maximal distance between the posterior aortic root and the posterior left atrium wall at end systole in the parasternal long-axis view [25]. LAE was defined as when the LAD was $\geq$3.9 cm in women and $\geq$4.1 cm in men, or $\geq$2.3 cm m^{-2} by body surface area (BSA) standardisation [24]. The calculation of BSA adopts the formula applicable to the Chinese population: BSA (m^2) = [0.0061 $\times$ height (cm) + 0.0124 $\times$ weight (kg) $-$ 0.0099] [26].

Famine exposure groups

Although food shortages gradually occurred in China from late 1958–1961, the accurate start and end date of the Chinese great famine is unknown. In the present study, famine exposure was grouped according to the birthdate of the selected candidates. According to previous studies [27,28], and aiming to reduce the classification error, subjects who were born between 1 October 1952 and 30 September 1964 were enrolled, whereas subjects

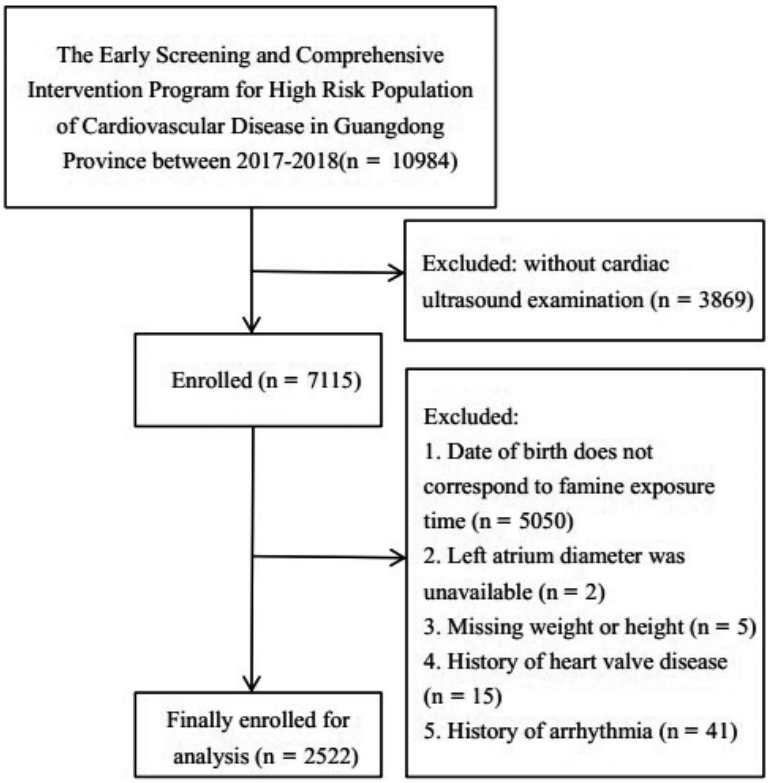

Figure 1 Research flow chart.

who were born between 1 October 1958 and 30 September 1959, as well those as born between 1 October 1961 and 30 September 1962, were excluded. Finally, candidates were classified into five groups including non-exposed (born between 1 October 1962 and 30 September 1964), fetal-exposed (born between 1 October 1959 and 30 September 1961), early-childhood exposed (born between 1 October 1956 and 30 September 1958), mid-childhood exposed (born between 1 October 1954 and 30 September 1956) and late childhood exposed (born between 1 October 1952 and 30 September 1954) groups.

Statistical analysis

All continuous variables are presented as the mean (SD) and categorical variables are presented as *n* (%). All baseline data were compared between the normal and LAE groups using Student's *t*-test or the Mann–Whitney *U*-test for continuous variables, with chi-squared tests for categorical variables. One-way analysis of variance was used for comparisons between multiple groups. Multivariate logistic regression with the crude and adjusted odds ratio (OR) and confidence interval (CI) was performed to investigate associations of exposure to famine during early life with the risk of LAE in adults. In adjusted models, gender, education level, marriage status, income, smoking status, drinking status, intake of vegetables and meat, physical activity, hypertension, diabetes, SBP, DBP, FBG, BMI, TG, TC, HDL-C, LDL-C, heart rate, and taking hypoglycaemic, antihypertensive and lipid-lowering drugs were adjusted. In addition, logistic regressions were stratified by gender, BMI, smoking, hypertension and diabetes to test for an interaction between famine exposure and LAE. $P < 0.05$ (two-sided) was considered statistically significant. All statistical analyses were performed using R, version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

Ethical approval

The survey protocol was approved by the Institutional Review Board of the Guangdong Provincial People's Hospital (No. GDREC2016438H (R2)). All participants provided their written informed consent.

Results

Baseline characteristics of participants

In the present study, 2808 subjects were enrolled, including 905 (35.9%) males. The mean (SD) age was 59.1 (3.65) years and mean (SD) LAD was 33.0 (4.17) mm. The basic characteristics of all participants are summarised in Table 1. There were significant differences between the LAE and without LAE

groups with respect to age, marriage status, education level, income, smoking status, combination with hypertension, currently taking antihypertensive drugs, SBP, HDL-C, heart rate and famine exposure (all $P < 0.05$).

In addition, the basic characteristics of study population according to exposure to the Chinese famine are shown in Table 2. In total, 392 (15.5%) subjects demonstrated LAE. The number of unexposed, fetal-exposed, early-childhood, mid-childhood and late childhood exposed groups was 510, 340, 487, 610 and 575, respectively. The prevalence of LAE among participants in the unexposed, fetal-exposed, early-childhood, mid-childhood and late childhood exposed groups was 55 (10.8%), 38 (11.2%), 88 (18.1%), 102 (16.7%) and 109 (19.0%), respectively. There were significant differences with respect to education level, smoking status, physical activity frequency, combination with hypertension, taking antihypertensive and lipid-lowering drugs, SBP, BMI, TC, LDL-C and LAD among the unexposed, fetal-exposed, early-childhood, mid-childhood and late childhood exposed groups (all $P < 0.05$).

Association of famine exposure in early life stage with left atrial enlargement in adulthood.

The association of famine exposure with the risk of LAE is demonstrated in Table 3. In model 1 without adjusting for variables, compared to the non-exposed group, fetal stage exposure appears to have no obvious relationship with LAE (OR = 1.040, 95% CI = 0.668–1.608, $P = 0.857$), although childhood exposure at any stage significantly increased the risk of LAE. After adjusting for potential covariates, the adjusted

Table 1 Baseline characteristics of participants with and without left atrial enlargement

	Overall (n = 2522)	Without LAE (n = 2130)	With LAE (n = 392)	P-value
Age (years)	59.1 (3.65)	58.9 (3.68)	59.9 (3.40)	<0.001
Gender, n (%)				
Male	905 (35.9%)	836 (39.2%)	69 (17.6%)	<0.001
Female	1617 (64.1%)	1294 (60.8%)	323 (82.4%)	
Married, n (%)	2318 (91.9%)	1970 (92.5%)	348 (88.8%)	0.017
Education level ≥ high school, n (%)	576 (22.8%)	504 (23.7%)	72 (18.4%)	0.025
Income > 50 000 yuan, n (%)	1286 (51.0%)	1103 (51.8%)	183 (46.7%)	0.071
Smoking, n (%)	472 (18.7%)	439 (20.6%)	33 (8.4%)	<0.001
Vegetable intake ≥3 days per week, n (%)	2231 (88.5%)	1884 (88.5%)	347 (88.5%)	0.999
Meat intake ≥3 days per week, n (%)	1519 (60.2%)	1265 (59.4%)	254 (64.8%)	0.053
Physical activity ≥3 days per week, n (%)	949 (37.6%)	814 (38.2%)	135 (34.4%)	0.149
Alcohol drinking, n (%)	128 (5.1%)	115 (5.4%)	13 (3.3%)	0.109
Hypertension, n (%)	1557 (61.7%)	1289 (60.5%)	268 (68.4%)	0.003
Diabetes, n (%)	492 (19.5%)	405 (19.0%)	87 (22.2%)	0.164
Hypoglycaemic drugs, n (%)	202 (8.0%)	164 (7.7%)	38 (9.7%)	0.216
Antihypertensive drugs, n (%)	770 (30.5%)	626 (29.4%)	144 (36.7%)	0.004
Lipid-lowering drug, n (%)	158 (6.3%)	129 (6.1%)	29 (7.4%)	0.371
SBP (mmHg)	143 (23.1)	142 (22.8)	149 (24.4)	<0.001
DBP (mmHg)	83.1 (12.4)	83.1 (12.3)	83.2 (13.1)	0.906
BMI (kg m^{-2})	24.8 (3.36)	24.8 (3.24)	25.0 (3.94)	0.372
TC (mmol L^{-1})	5.82 (1.50)	5.81 (1.50)	5.86 (1.50)	0.572
TG (mmol L^{-1})	1.95 (1.09)	1.95 (1.09)	1.96 (1.11)	0.804
LDL-C (mmol L^{-1})	3.38 (1.25)	3.38 (1.24)	3.32 (1.27)	0.390
HDL-C (mmol L^{-1})	1.63 (0.468)	1.62 (0.461)	1.73 (0.491)	<0.001
FBG (mmol L^{-1})	6.12 (1.85)	6.11 (1.86)	6.18 (1.74)	0.466
Heart rate (beats min^{-1})	79.8 (11.6)	80.1 (11.5)	77.7 (12.0)	<0.001
LAD (mm)	33.0 (4.17)	32.1 (3.52)	37.8 (4.24)	<0.001
Famine exposure, n (%)				
Non-exposed	510 (20.2%)	455 (21.4%)	55 (14.0%)	<0.001
Fetal exposed	340 (13.5%)	302 (14.2%)	38 (9.7%)	
Early-childhood exposed	487 (19.3%)	399 (18.7%)	88 (22.4%)	
Mid-childhood exposed	610 (24.2%)	508 (23.8%)	102 (26.0%)	
Late childhood exposed	575 (22.8%)	466 (21.9%)	109 (27.8%)	

Data are presented as the mean (SD) or as a percentage.

BMI, body mass index; DBP, diastolic blood pressure; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; LAD, left atrium diameter; LAE, left atrial enlargement; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

Table 2 Basic characteristics of study population according to exposure to the Chinese famine

	Overall (n = 2522)	Non-exposed (n = 510)	Fetal exposed (n = 340)	Early-childhood exposed (n = 487)	Mid-childhood exposed (n = 610)	Late childhood exposed (n = 575)	P-value
Birthdate	/	1962/10/1–1964/9/30	1959/10/1–1961/9/30	1956/10/1–1958/9/30	1954/10/1–1956/9/30	1952/10/1–1954/9/30	
Gender, *n* (%)							
Male	905 (35.9%)	182 (35.7%)	101 (29.7%)	166 (34.1%)	234 (38.4%)	222 (38.6%)	0.043
Female	1617 (64.1%)	328 (64.3%)	239 (70.3%)	321 (65.9%)	376 (61.6%)	353 (61.4%)	
Married, *n* (%)	2318 (91.9%)	480 (94.1%)	312 (91.8%)	451 (92.6%)	552 (90.5%)	523 (91.0%)	0.197
Education level ≥ high school, *n* (%)	576 (22.8%)	122 (23.9%)	101 (29.7%)	134 (27.5%)	114 (18.7%)	105 (18.3%)	<0.001
Income> 50 000 yuan, *n* (%)	1286 (51.0%)	263 (51.6%)	178 (52.4%)	249 (51.1%)	327 (53.6%)	269 (46.8%)	0.194
Smoking, *n* (%)	472 (18.7%)	102 (20.0%)	54 (15.9%)	79 (16.2%)	135 (22.1%)	102 (17.7%)	0.052
Vegetable intake ≥3 days per week, *n* (%)	2231 (88.5%)	440 (86.3%)	294 (86.5%)	436 (89.5%)	546 (89.5%)	515 (89.6%)	0.227
Meat intake ≥3 days per week, *n* (%)	1519 (60.2%)	308 (60.4%)	211 (62.1%)	306 (62.8%)	350 (57.4%)	344 (59.8%)	0.415
Physical activity ≥3 days per week, *n* (%)	949 (37.6%)	166 (32.5%)	131 (38.5%)	205 (42.1%)	218 (35.7%)	229 (39.8%)	0.018
Alcohol drinking, *n* (%)	128 (5.1%)	27 (5.3%)	17 (5.0%)	26 (5.3%)	29 (4.8%)	29 (5.0%)	0.992
Hypertension, *n* (%)	1557 (61.7%)	278 (54.5%)	188 (55.3%)	307 (63.0%)	382 (62.6%)	402 (69.9%)	<0.001
Diabetes, *n* (%)	492 (19.5%)	99 (19.4%)	61 (17.9%)	107 (22.0%)	102 (16.7%)	123 (21.4%)	0.151
Hypoglycaemic drugs, *n* (%)	202 (8.0%)	31 (6.1%)	24 (7.1%)	40 (8.2%)	45 (7.4%)	62 (10.8%)	0.052
Antihypertensive drugs, *n* (%)	770 (30.5%)	108 (21.2%)	84 (24.7%)	158 (32.4%)	199 (32.6%)	221 (38.4%)	<0.001
Lipid-lowering drug, *n* (%)	158 (6.3%)	17 (3.3%)	19 (5.6%)	24 (4.9%)	46 (7.5%)	52 (9.0%)	0.001
SBP (mmHg)	143 (23.1)	140 (24.0)	141 (22.2)	144 (22.3)	144 (22.3)	147 (23.9)	<0.001
DBP (mmHg)	83.1 (12.4)	84.2 (13.1)	83.3 (13.0)	83.2 (12.3)	82.2 (11.8)	83.1 (12.2)	0.053
BMI (kg m^{-2})	24.8 (3.36)	25.2 (3.38)	25.1 (3.38)	24.7 (3.49)	24.5 (3.27)	24.9 (3.26)	0.011
TC (mmol L^{-1})	5.82 (1.50)	5.93 (1.45)	5.98 (1.51)	5.80 (1.48)	5.77 (1.50)	5.69 (1.54)	0.001
TG (mmol L^{-1})	1.95 (1.09)	2.02 (1.14)	1.91 (1.14)	1.92 (1.05)	1.87 (1.04)	2.01 (1.11)	0.660
LDL-C (mmol L^{-1})	3.38 (1.25)	3.50 (1.27)	3.49 (1.23)	3.34 (1.20)	3.37 (1.23)	3.24 (1.27)	0.004
HDL-C (mmol L^{-1})	1.63 (0.468)	1.62 (0.471)	1.68 (0.484)	1.65 (0.475)	1.64 (0.457)	1.60 (0.459)	0.394
FBG (mmol L^{-1})	6.12 (1.85)	6.07 (1.97)	6.01 (1.63)	6.17 (1.86)	5.98 (1.44)	6.32 (2.17)	0.086
LAD (mm)	33.0 (4.17)	32.6 (4.36)	32.7 (3.68)	33.1 (3.80)	32.9 (4.07)	33.5 (4.60)	0.001
Heart rate (beats min^{-1})	79.8 (11.6)	80.3 (11.1)	80.3 (11.1)	79.5 (11.6)	80.1 (12.4)	78.9 (11.4)	0.058
LAE, *n* (%)	392 (15.5%)	55 (10.8%)	38 (11.2%)	88 (18.1%)	102 (16.7%)	109 (19.0%)	<0.001

Data are presented as the mean (SD) or as a percentage.

BMI, body mass index; DBP, diastolic blood pressure; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; LAD, left atrium diameter; LAE, left atrial enlargement; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride

ORs and 95% CI of LAE were OR = 0.956, 95% CI = 0.605–1.500 (P = 0.847); OR = 1.630, 95% CI = 1.116–2.399 (P = 0.012); OR = 1.647, 95% CI = 1.140–2.403 (P = 0.008); and OR = 1.748, 95% CI = 1.208–2.555 (P = 0.003), respectively.

Association of famine exposure with left atrial enlargement by subgroups

As shown in Table 4, when stratified by gender, only in the female population did exposure to famine in early, mid and late childhood increase the risk of LAE in adulthood, whereas, in males, exposure at any stage during early life showed no association with LAE in adults. Similar results were found for non-smokers and the diabetes subgroup. In the BMI subgroup, only exposure in middle and late childhood increased the risk of adult LAE for the group with a BMI ≥24 kg m^{-2}. For the hypertensive subgroup, in the hypertensive population, only middle-childhood exposure (OR = 1.736, 95% CI = 1.097–2.794) was associated with LAE, whereas, in the non-hypertensive population, only late childhood exposure (OR = 2.407,

Table 3 Risk of left atrial enlargement among four exposed groups compared to the non-exposed group

	Model 1		Model 2	
Groups	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Non-exposed	1.00 (ref)		1.00 (ref)	
Fetal exposed	1.040 (0.668–1.608)	0.857	0.956 (0.605–1.500)	0.847
Early-childhood exposed	1.824 (1.273–2.634)	0.001	1.630 (1.116–2.399)	0.012
Mid-childhood exposed	1.661 (1.174–2.373)	0.005	1.647 (1.140–2.403)	0.008
Late childhood exposed	1.935 (1.371–2.759)	<0.001	1.748 (1.208–2.555)	0.003

Data are presented as the odds ratio (OR) and 95% confidence interval (CI).
Model 1 with no variable was adjusted.
Model 2 with gender, education and marital status, income, smoking status, drinking status, intake of vegetables and meat, physical activity, hypertension, diabetes, systolic and diastolic blood pressure, fasting blood glucose, body mass index, triglycerides, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, heart rate, taking hypoglycaemic, antihypertensive and lipid-lowering drugs were adjusted.

95% CI = 1.267–4.660) was associated with LAE. In addition, we found that there was statistically significant interaction between the effects of famine exposure and diabetes on LAE ($P_{\text{interaction}} = 0.007$) and this effect had no obvious interaction with other variables, including gender, smoking, BMI and hypertension (all $P_{\text{interaction}} > 0.05$).

Discussion

The present study demonstrates for the first time that famine exposure during early, middle and late childhood is significantly linked with an increased risk of LAE in adult life, although exposure to famine at the fetal stage is not associated with LAE. After stratification by gender, we observed that famine exposure in early life increased the risk of LAE in adulthood only in females. The effect of famine exposure on LAE was affected by the interaction with diabetes status.

The Early Life Origin Theory postulates the prenatal and early developmental origin of adult onset disease and also highlights the importance of the maternal environment [29]. Our finding revealed that, compared to non-exposure to famine in early life, fetal stage exposure did not increase the risk of LAE, whereas early, middle and late childhood exposure can increase the risk of LAE by 63%, 65% and 75%, respectively. Although the mechanism of nutritional status and LAE is still unclear, there are some potential mechanisms explaining the association between famine exposure in early life and LAE in adulthood. First, food shortages can lead to a lack of nutrition. Basic research has shown that dietary intervention could reduce LAE in dogs [11]. Second, famine exposure during early life resulted in individuals being more prone to obesity, insulin resistance, diabetes and hypertension as adults [14,29]. Shigematsu *et al.* [7] reported that left atrial size was influenced by insulin resistance and obesity. Ou *et al.* [5] suggested that high SBP, high BMI and diabetes were risk factors for LAE. Finally, it has been revealed that there is a significance association between under-nutrition and an increased hypothalamic-pituitary-adrenal response to psychological stress [30]. When stress occurs, a high metabolic state exists that can cause a significant increase in the stroke volume of the heart and lead to compensatory or decompensated changes in cardiac structure and function. Eventually, decompensated cardiac chamber enlargement or decreased diastolic dysfunction appear [31].

In addition, the gender subgroup found that, with exposure to famine in early life, women had a higher risk of LAE in adulthood than men. There are several possible reasons for this. On the one hand, as a result of the 'preference for sons' in some parts of China in the past, women may have suffered more severe malnutrition. On the other hand, because oestrogen has a certain protective effect on the cardiovascular system, the neuroendocrine system in women was affected after famine, so that hormone secretion was also affected after adulthood. It has been shown that the diffuse neuroendocrine system may play a critical role in the adaptation to impending famine [32]. In the subgroups of BMI, smoking, hypertension and diabetes, we also found that the effects of famine exposure on LAE were different at different stages of early life, and the effect of famine on LAE was only modified by adult diabetes status. The reasons for these results still remain unclear.

The present study is the the first to report that exposure to famine during early life in the Chinese population is associated with LAE in adulthood. However, some shortcomings of the present study should also be considered. First, although the present study was grouped according to date of birth, age still has an effect on the results. Second, this was a cross-sectional study with a relatively small sample, and the results cannot be

Table 4 Association of famine exposure and left atrial enlargement by subgroups

Subgroup	Cases/ total	Non-exposed	Fetal exposed OR (95% CI)	Early-childhood exposed OR (95% CI)	Mid-childhood exposed OR (95% CI)	Late childhood exposed OR (95% CI)	*P*-interaction
Gender							
Male	69/905	1.00 (ref)	1.222 (0.390–3.551)	2.006 (0.850–4.935)	1.369 (0.585–3.345)	1.693 (0.761–3.996)	0.474
Female	323/1617	1.00 (ref)	0.923 (0.556–1.520)	1.586 (1.037–2.450)	1.736 (1.149–2.654)	1.772 (1.165–2.725)	
BMI (kg m^{-2})							
≥24	210/1455	1.00 (ref)	1.128 (0.616–2.047)	1.540 (0.906–2.639)	1.922 (1.169–3.214)	1.998 (1.214–3.346)	0.883
<24	179/1059	1.00 (ref)	0.889 (0.423–1.827)	1.694 (0.953–3.076)	1.424 (0.804–2.579)	1.609 (0.898–2.941)	
Smoking							
Yes	33/472	1.00 (ref)	2.214 (0.459–10.166)	2.400 (0.602–10.126)	2.576 (0.760–9.910)	2.314 (0.656–9.023)	0.507
No	359/2050	1.00 (ref)	0.904 (0.557–1.455)	1.622 (1.089–2.438)	1.655 (1.120–2.470)	1.762 (1.192–2.634)	
Hypertension							
Yes	268/1557	1.00 (ref)	1.008 (0.561–1.792)	1.568 (0.971–2.567)	1.736 (1.097–2.794)	1.540 (0.975–2.475)	0.280
No	124/965	1.00 (ref)	0.861 (0.399–1.813)	1.642 (0.863–3.170)	1.397 (0.731–2.706)	2.407 (1.267–4.660)	
Diabetes							
Yes	87/492	1.00 (ref)	1.193 (0.480–2.920)	0.913 (0.406–2.057)	0.932 (0.410–2.128)	0.839(0.381–1.868)	0.007
No	305/2030	1.00 (ref)	0.889 (0.513–1.524)	1.974 (1.269–3.111)	1.918 (1.250–2.988)	2.165 (1.411–3.375)	

Data are presented as the odds ratio (OR) and 95% confidence interval (CI).

P values are for the comparison of the difference in subgroup condition.

BMI, body mass index.

Gender, education and marital status, income, smoking status, drinking status, intake of vegetables and meat, physical activity, hypertension, diabetes, systolic and diastolic blood pressure, fasting blood glucose, BMI, triglycerides, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, heart rate, taking hypoglycaemic, antihypertensive and lipid-lowering drugs were adjusted.

extended to the entire Chinese population. Third, some of the baseline information was self-reported and there may have beeen recall bias. Fourth, the exact time of famine was not clear, and there may be inaccurate grouping. In addition, the present study only analysed the current surviving population, and so it may underestimate or overestimate the role of famine in LAE.

In conclusion, famine exposure during childhood was found to be significantly associated with LAE in adulthood, although there was a difference with respect to gender, which indicated that malnutrition during early life may play a vital role in the change of cardiac structure in adults. The relationship between famine exposure in early life and LAE during late life was altered by diabetes status in adulthood. Ensuring proper and sufficient nutrition in the early life not only enabled infants and young children to grow up healthy, but also prevented the occurrence of related cardiovascular diseases, including LAE in adulthood. The mechanism of famine exposure and LAE is still unclear, and additional research is required for clarification.

Transparency declaration

The lead author confirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with STROBE guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

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Conflict of interests, source of funding, authorship

The authors declare that they have no conflicts of interest.

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ZYL and FYQ conceived the study. LL and HYQ wrote the manuscript. LL and HYQ performed the statistical analyses. LK, ZYL and FYQ contributed to the critical review of the manuscript. CCL, YYL and HJY participated in the acquisition of the data. FYQ had full

access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final version of the manuscript submitted for publication.

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EARLY LIFE NUTRITION

Persistent inflammation, immunosuppression and catabolism syndrome (PICS) in critically ill children is associated with clinical outcomes: a prospective longitudinal study

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Abstract

Background: Persistent inflammation, immunosuppression and catabolism syndrome (PICS) has been described in critically ill adults and may contribute to unfavourable outcomes. The present study aimed to describe and characterise PICS in critically ill children (PICS-ped) and to verify its association with clinical outcomes.
Methods: A prospective longitudinal study was conducted in a paediatric intensive care unit (PICU) with children aged between 3 months and 15 years. PICS-ped, based on adult definition, was described. PICS-ped was defined as PICU length of stay >14 days; C-reactive protein > 10.0 mg L^{-1}; lymphocytes <25%; and any reduction of mid-upper arm circumference *Z*-score. Clinical, demographic, nutritional status, nutrition therapy parameters and clinical outcomes were assessed. Statistical analysis comprised Mann–Whitney and Fisher's chi-squared tests, as well as logistic and Cox regression. $P < 0.05$ was considered statistically significant.
Results: In total, 153 children were included, with a median age of 51.7 months (interquartile range 15.6–123.4 months), and 60.8% male. The mortality rate was 10.5%. The prevalence of PICS-ped was 4.6%. Days using vasoactive drugs and days using antibiotics were associated with PICS-ped. PICS-ped was associated with mortality in crude (odds ratio = 6.67; $P = 0.013$) and adjusted analysis (odds ratio = 7.14; $P = 0.017$). PICS-ped was also associated with PICU and hospital length of stay, as well as duration of mechanical ventilation. Similar results were found in a subset of critically ill children who required mechanical ventilation for more than 48 h.
Conclusions: Children with PICS-ped required antibiotics or vasoactive drugs for a longer period. PICS-ped was associated with poor clinical outcomes in critically ill children. More studies are needed to properly define PICS-ped for this population.

Introduction

After a major insult such as trauma or sepsis, critically ill patients may experience both the systemic inflammatory response syndrome and the compensatory anti-inflammatory response syndrome. These situations can lead to an early multiple organ dysfunction syndrome and death, recovery or chronic critical illness (CCI). CCI is

characterised by prolonged stay in an intensive care unit (ICU) (>14 days), low-grade organ dysfunction and multiple phenotypes, including chronic long-term inflammation, immunosuppression and catabolism [1].

Therefore, patients who survive the acute phase and develop CCI show a phenotype called persistent inflammation, immunosuppression, and catabolism syndrome (PICS), which recently has been described in critically ill adults. The most common biomarkers that describe this syndrome in adults are: inflammation: C-reactive protein (CRP) >50 μg dL^{-1} (0.5 mg L^{-1}), retinol-binding protein <1 mg dL^{-1}; immunosuppression – total lymphocyte count <0.80 × 10^9; and protein catabolism – serum albumin <3.0 g dL^{-1}, creatinine height index <80%, weight loss (Fig. 1) [2]. These biomarkers are not direct measurements of inflammation, immunosuppression or protein catabolism, although they are considered as surrogates available in most services [3].

As a result of protein catabolism, adult patients with PICS have increased susceptibility to nosocomial infections, which leads to more inflammation and resumption of the vicious cycle [2]. Critically ill children are at high risk of loss of lean mass and poor clinical outcomes [4]. In response to stress, inflammatory mediators increase, whereas nutritional status deteriorates [5]. During the critical illness, there is an activation of the immune system, characterised by an exacerbation of pro-inflammatory response, which is associated with loss of lean body tissue and proteolysis [6]. Also, critically ill children with sepsis have early innate and adaptive immune suppression, which is associated with longer periods of organ dysfunction [7].

We hypothesise that PICS in paediatrics (PICS-ped) exists in critically ill and children with PICS-ped have different characteristics compared to critically ill children without PICS-ped. In addition, PICS-ped is associated with longer hospital length of stay (LOS) and with a longer duration of mechanical ventilation (MV). Therefore, considering that PICS-ped has not been evaluated and properly defined in critically ill children, the present study aimed to describe and characterise PICS-ped for critically ill children based on the published adult definition, as well as to verify its association with hospital LOS and duration of MV in an exploratory study.

Materials and methods

Study design and participants

A prospective single-centre cohort study was conducted between July 2013 and January 2016. Critically ill children, aged 3 months to 15 years old, admitted for at least 48 h to medical and surgical PICU in a state in Southern Brazil, were included. Exclusion criteria were death within 72 h of admission and oral nutrition therapy. A non-probability sampling process by time saturation was applied. The study was approved by the local Institutional Review Board (Human Research Ethics Committee) (402.469). Informed consent was obtained from the parents or guardians of all the patients enrolled in the study.

Persistent inflammation, immunosuppression and catabolism syndrome in the paediatric population

To our knowledge, PICS for children has not been defined yet. Therefore, based on the definition proposed for adults, [3] variables that are more frequently used in the clinical practice were selected to identify patients with

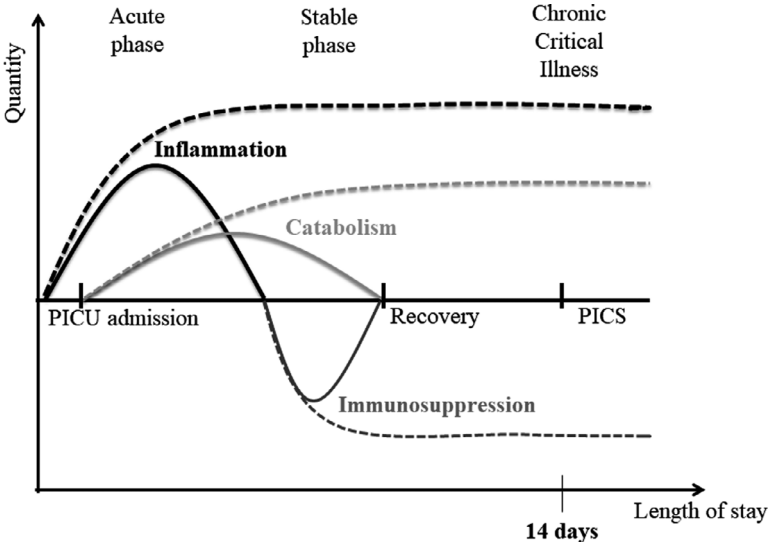

Figure 1 Variables that define persistent inflammation, immunosuppression and catabolism syndrome (PICS) in critical illness. Inflammation: ↑ C-reactive protein; ↑ retinol-binding protein. Immunosuppression: ↓ lymphocyte count. Catabolism: ↓ serum albumin; weight loss and creatinine height index. Bold line: inflammation; grey line: catabolism; dark grey line: immunosuppression. Dotted line: PICS patient; solid line: regular patients. Adapted with permission from Gentile *et al*. (2012).

PICS-ped. To define the cut-off of PICU LOS for CCI, a receiver operating characteristic curve was constructed, using as an outcome the overall mortality. Similarly to the adult definition, it was determined the cut-off ≥14 days (area under the curve = 0.70; 95% CI = 0.59–0.82; sensitivity 50% and specificity 85%).

For persistent inflammation, hs-CRP (mg L^{-1}) >10.0 mg L^{-1} at day 14 was used, based on a reference value for acute-phase inflammatory responses [8]. Lymphocytes <25% on day 14 were used as biomarkers for immunosuppression [9]. Protein catabolism was defined as any decrease in the mid-upper arm circumference-for-age *Z*-score (MUACz) after 14 days. Weight and body mass index (BMI) were not used as a result of limitations of weight measurement in this population, nor albumin because of inflammation bias [10]. CRP, lymphocytes and MUAC were also measured at PICU admission. To minimise the inter-variability in the anthropometric measures, MUAC was recorded by a trained researcher or a dietitian and the same researcher assessed the measure on day 1 and day 14. The PICS-ped is described in Table 1. To be classified with PICS-ped, the child should meet all four criteria.

Demographic and clinical characteristics

At admission, the Pediatric Index of Mortality 2 (PIM2) was calculated and expressed as the probability of death [11]. The reason for admission was classified as medical or surgical. Complex Chronic Condition (CCC) was assessed [12]. The duration (in days) of vasoactive drugs and antibiotics was assessed. The dose and the duration of vasoactive drugs and antibiotics were defined by the local staff based on the daily clinical condition of the patient. Fluid overload (in the first 3 days) was calculated and expressed as a percentage. Nosocomial infections, duration of MV, PICU and hospital LOS, and overall mortality were recorded in the patient chart. Nosocomial infection was considered as any acquired infection: bloodstream, urinary tract or pneumonia after 48 h of PICU admission [13]. Overall mortality was defined as PICU and hospital mortality combined.

Table 1 Criteria for persistent inflammation, immunosuppression and catabolism syndrome in pediatric patients (PICS-ped)

PICS-ped criteria	Variable	Cut-off
Chronic critical illness	PICU length of stay (days)	>14 days*
Persistent inflammation	C-reactive protein (mg L^{-1})	>10 mg L^{-1} after 14 days
Immunosuppression	Lymphocytes (% of total leucocytes)	<25% after 14 days
Catabolism	Mid-upper arm circumference (*Z*-score)	Any reduction in 14 days

PICU, pediatric intensive care unit.
*Area under the curve = 0.70; confidence interval 95% 0.59; 0.82; sensibility 50%; specificity 85%.

Nutritional status

Weight, height, and MUAC were measured within 72 h of admission and repeated weekly until the patient's discharge, in accordance with World Health Organization (WHO) methodology [14]. Weight was measured on a paediatric scale (BP Baby; Filizola, São Paulo, Brazil), with a precision of 1 g. Length/height was measured by an anthropometer with a precision of 0.1 cm and, when height was not feasible, for children ≥6 years old, it was predicted based on knee height [15] The *Z*-scores for body mass index-for-age (BMIz) and height-for-age (HAz) were calculated using ANTHRO or ANTHRO-PLUS (WHO, Geneva, Switzerland). MUAC was measured by a previously trained professional with a flexible inelastic tape (cm), with a precision of 0.1 cm, at the midpoint between the acromion and the olecranon. The *Z*-score for MUACz was calculated in accordance with WHO values in children <5 years old, and according to Frisancho [16] for children >5 years old. Nutritional status was classified as moderate undernutrition (≤–2 *Z*-score), mild undernutrition (≤–1 *Z*-score) and eutrophic (>−1 *Z*-score) [17].

Biochemical parameters

Biochemical parameters were measured within 72 h of admission and repeated weekly until patient discharge. Serum albumin was assessed using the bromocresol green method, with Kit Quimialb – Albumin (EBRAM Ltda, São Paulo, Brazil) using the automated equipment QUIMISAT 450 (EBRAM Ltda).

Serum high-sensitive CRP (hs-CRP) (mg L^{-1}) was determined using the latex immunoturbidimetric method with a commercial kit (Turb – PCR; EBRAM Ltda). The hs-CRP/albumin ratio, obtained as the ratio between the hs-CRP concentration and the albumin concentration, was expressed as mg L^{-1} : g dL^{-1}. The hemogram was analysed by the semi-automated method using Heco 5 Plus equipment (Radim Company, Pomezia, Italy) and the values of leukocyte, neutrophil and lymphocytes were expressed in cells $mm^{-3} \times 10^3$.

Nutrition therapy

Nutrition therapy variables included time to initiate nutrition therapy, route of delivery, and actual energy

and protein intake during the PICU stay. Early nutrition therapy (enteral and/or parenteral nutrition) was defined as initiation within 24 h after PICU admission.

Outcomes

The main outcomes were hospital LOS and duration of MV.

Statistical analysis

Statistical data analysis was performed using STATA, version 11.0 (Stata Corp., College Station, TX, USA). Categorical variables were described in absolute values and frequency. Quantitative variables were reported as the median and interquartile range (IQR).

Crude and adjusted for PIM2 logistic regression analyses were performed to explore the effect of PICS-ped on the overall mortality. The results were expressed as odds ratio (OR) and 95% confidence intervals (95% CI). To explore variables associated with PICS-ped, Fisher's chi-square and Mann–Whitney tests were applied. To assess the influence of PICS-ped on the duration of MV, PICU and hospital LOS, crude and adjusted Cox regressions were used, and the results were expressed as hazard ratio (HR) and 95% CI. The association with clinical outcomes was also tested in a subset of critically ill children who required MV for more than 48 h, excluding children with potentially milder disease. $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

Between July 2013 and January 2016, 715 critically ill children were admitted to the PICU. Of these, 591 were eligible and 153 were included in the study. A flowchart of the recruitment of the participants is provided in the Supporting information (Fig. S1). The median age was 51.7 months (IQR = 15.6–123.4), 60.8% were male and the median PIM2 was 4.7% (IQR = 1.3–16.0). Patients were mainly admitted for medical diagnostics (73.9%). Mortality was observed in 10.5% of the cohort. Clinical and nutrition characteristics are shown in Table 2. The median MUACz reduction after 14 days was a *Z*-score of −1.02 (IQR = –1.56 to −0.75).

Table 2 Characterisation of critically ill children in a pediatric intensive care unit (n = 153)

Variables	Median [IQR]/*n* (%)
Sex (female)	60 (39.2)
Age (months)	51.7 [15.6–123.4]
Reason of admission	
Medical	113 (73.9)
Surgical	40 (26.1)
Sepsis/septic shock	29 (18.9)
Burn	5 (3.3)
Trauma	18 (11.8)
PIM2 (%)	4.7 [1.3–16.0]
C-reactive protein (mg L^{-1})	33.1 [8.4–66.5]
Lymphocytes (%)	14.6 [8.0–25.0]
Nutritional status at admission	
BMI/A *Z*-score (n = 144)	−0.02 [−1.24 to 0.94]
Mild undernutrition	21 (14.6)
Moderate or severe undernutrition	22 (15.3)
Height-for-age *Z*-score (n = 144)	−0.84 [−1.94 to 0.33]
Mild undernutrition	32 (22.2)
Moderate or severe undernutrition	34 (23.6)
MUAC/A *Z*-score (n = 140)	0.04 [−1.12–1.00]
Mild undernutrition	27 (19.3)
Moderate or severe undernutrition	13 (9.3)
Early NT	95 (62.9)
Clinical outcomes	
Nosocomial infection (n = 149)	36 (24.2)
Hospital LOS (days) (n = 149)	21 [13; 35]
PICU LOS (days)	7 [4–12]
Duration of MV (days) (n = 124)	4.5 [3–10]
Overall mortality	16 (10.5)

BMI/A, body mass index-for-age; IQR, interquartile range; LOS, length of stay; MUAC/A, mid-upper arm circumference-for-age; MV, mechanical ventilation; NT, nutrition therapy; PICU, pediatric intensive care unit; PIM2, Pediatric Index of Mortality 2.
Early NT: enteral and/or parenteral nutrition initiated within 24 h after PICU admission.
Mild undernutrition <−1 *Z*-score; Moderate/severe undernutrition <−2 *Z*-score.

Variables associated with persistent inflammation, immunosuppression and catabolism syndrome in critically ill children

The prevalence of PICS-ped was 4.6% (n = 7). The prevalence of CCC in patients without PICS-ped was 27.7%, whereas no patient with PICS-ped had CCC. However, a higher prevalence of CCC was observed in surgical patients (42.5%) compared to clinical patients (22.1%) (P = 0.013; data not shown). Table 3 shows the comparison of variables between patients with and without PICS-ped. Days using vasoactive drugs and days using antibiotics were associated with PICS-ped. There were no significant differences between patients with and without PICS-ped regarding nutrition therapy within the first 7 days (Table 3).

Association between persistent inflammation, immunosuppression and catabolism syndrome in critically ill children and clinical outcomes

PICS-ped was associated with mortality in crude (OR = 6.67; P = 0.013) and adjusted analysis (OR = 7.14;

Table 3 Characterisation of critically ill children in a pediatric intensive care unit stratified by PICS-ped (n = 153)

Variables	Median [IQR]/*n* (%)		
	without PICS-ped (n = 146)	with PICS-ped (n = 7)	*P*-value
Sex (female) *n* (%)	56 (38.36)	4 (57.14)	0.343†
Age (months)	52.6 [15.5–123.4)	43.8 [16.2–123.7]	0.885‡
Reason for admission *n* (%)			
Medical	109 (74.7)	4 (57.1)	0.379†
Surgical	37 (25.3)	3 (42.9)	
Respiratory insufficiency *n* (%)	46 (31.5)	3 (42.9)	0.681†
PIM2 (%)	4.6 [1.2–15.9]	12 [3.8–44.9]	0.188‡
Vasoactive drugs (days)	2.0 [1.0–6.0]	19.5 [10.0–36.0]	<0.001‡
Antibiotics (days)	5.0 [3.0–9.0]	24.0 [170–35.0]	<0.001‡
Oedema at PICU admission *n* (%)	15 (10.3)	1 (14.29)	0.546†
Fluid overload* (%)	66.3 [35.1–114.0]	97.0 [51.0–151.1]	0.324‡
Biochemical parameters at PICU admission			
Albumin (g dL^{-1}) (n = 147)	3.00 [2.50–3.45]	2.70 [2.30–3.20]	0.285‡
C-reactive protein (mg L^{-1}) (n = 143)	29.45 [8.35–66.25]	63.10 [36.50–89.30]	0.155‡
C-reactive protein/albumin (g L^{-1} : mg dL^{-1}) (n = 139)	10.63 [2.61–25.81]	27.43 [14.04–27.91]	0.165‡
Lymphocytes (%) (n = 144)	14.6 [7.8–25.1]	15.0 [12.0–18.0]	0.996‡
Nutritional status at PICU admission			
BMIz <−2 *Z*-score *n* (%)	22 (16.1)	0 (0.00)	–
MUACz <−2 *Z*-score *n* (%)	11 (8.3)	2 (28.57)	0.128†
MUACz reduction in 14 days (*Z*-score) (n = 18)	−1.08 (−1.53 to −0.82)	−0.97 (−1.65 to −0.35)	1.000‡
Nutrition Therapy			
Early NT *n* (%)	91 (63.2)	4 (57.1)	0.711†
Route of NT (n = 139) *n* (%)			
Enteral only	108 (81.8)	6 (85.7)	1.000†
Parenteral + enteral or Parenteral	24 (18.2)	1 (14.3)	
Energy intake			
Goal (kcal kg^{-1} day^{-1})§	48.2 (36.4–56.9)	51.3 (39.5–54.8)	0.978‡
Prescribed within 7 days (kcal kg^{-1} day^{-1})	33.1 (19.5–47.3)	31.4 (29.6–45.4)	0.766‡
Actual energy intake within 7 days (kcal kg^{-1} day^{-1})	29.0 (14.9–41.7)	25.3 (22.0–41.4)	0.618‡
Prescribed within 8–14 days (kcal kg^{-1} day^{-1})	47.2 (35.7–65.3)	58.7 (54.1–65.9)	0.060‡
Actual energy intake 8–14 days (kcal kg^{-1} day^{-1})	42.5 (29.7–51.2)	46.6 (40.8–59.4)	0.131‡
Protein intake¶			
Prescribed within 7 days (g kg^{-1} day^{-1})	1.00 (0.67–1.48)	1.17 (0.79–1.54)	0.600‡
Actual protein intake within 7 days (g kg^{-1} day^{-1})	0.86 (0.47–1.21)	1.02 (0.64–1.54)	0.512‡
Prescribed within 8–14 days (g kg^{-1} day^{-1})	1.76 (1.17–1.95)	1.94 (1.48–2.32)	0.142‡
Actual protein intake 8–14 days (g kg^{-1} day^{-1})	0.96 (0.51–1.53)	1.54 (0.89–1.98)	0.050‡
Clinical outcomes			
Nosocomial infection *n* (%)	33 (23.2)	3 (42.9)	0.360†
Duration of MV n = 125 (days)	4 (3–8)	26 (20–40)	<0.001‡
PICU LOS (days)	6 (4–11)	29 (21–40)	<0,001‡
Hospital LOS (days)	20 (13–31)	59 (45–142)	<0.001‡

BMIz, body mass index-for-age; LOS, length of stay; MV, mechanical ventilation; MUACz, mid-upper arm circumference-for-age; NT, nutrition therapy; PICU, pediatric intensive care unit; PIM2, Pediatric Index of Mortality 2.

Early NT: enteral and/or parenteral nutrition initiated within 24 h after PICU admission.

*on the first 3 days.

†Fisher's chi-squared.

‡Mann–Whitney test.

§Based on the Schofield equation.

¶Protein goal was defined as 1.5 g kg^{-1} day^{-1}.

P = 0.017). After adjustment for PIM2, patients with PICS-ped had lower chance of an earlier hospital discharge (HR = 0.22; 95% CI = 0.08; 0.61; P = 0.003) and extubation (HR = 0.21; 95% CI = 0.07; 0.57; P = 0.003). Similar results were found in a subset of critically ill children who required MV for more than 48 h (Fig. 2).

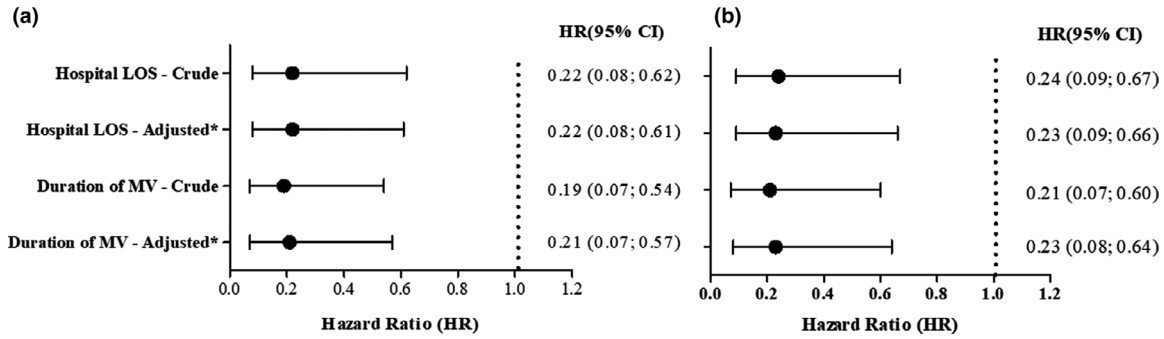

Figure 2 Association of persistent inflammation, immunosuppression and catabolism syndrome in pediatrics (PICS-ped) and clinical outcomes in critically ill children (PICS-ped). (a) All children (n = 153). (b) Children with MV >48 h (n = 125). CI, confidence interval; LOS, length of stay; MV, mechanical ventilation.

Discussion

PICS-ped (defined as PICU LOS >14 days; CRP> 10.0 mg L^{-1} after 14 days; lymphocytes <25% after 14 days; reduction of MUACz after 14 days) was observed in 4.58% of the critically ill children. These children required antibiotics or vasoactive drugs for a longer period. PICS-ped was associated with poor clinical outcomes. Our results complement existing knowledge regarding PICS and highlight the importance of the early identification of children that are at risk of PICS-ped. By identifying factors that may contribute to PICS condition and characterise this subset of critically ill children, it is possible to recognise conditions that increase the risk for PICS-ped. Therefore, it could improve both early detection and strategies that focus on the treatment of these potential risk factors.

The term PICS is a proposed definition for the persistent inflammation, immunosuppression and catabolism framework that occurs in CCI patients [3]. The prolonged PICU LOS may increase the risk for mortality in critically ill children by up to five-fold [18]. There is no consensus of the CCI definition for PICU and, consequently, few studies have used it as an outcome for this population [19]. Studies have reported that the definition of CCI in the paediatric population ranges from ≥12 days to 30 days. More recently, studies have suggested a definition for CCI in PICU that includes ≥14 consecutive days; or who have a history of prolonged PICU stay and ≥ 2 acute care/PICU admissions within 12 months [18]. In an observational study with 1629 critically ill children, the prevalence of prolonged PICU LOS (>14 days) was 19.6% and the mortality rate was 14.7% versus 11.1% in patients with no prolonged PICU LOS [20]

Patients who progress to CCI usually show signs of a persistent inflammatory response and immunosuppression. In adults, based on genomic analysis, the current evidence suggests that critically ill patients with complicated clinical outcomes exhibit a persistent genomic expression change with defects in the adaptive immune response and increased inflammation [21]. Critically ill children with septic shock exhibit early adaptive immunosuppression, which is associated with poor outcomes [22]. In a study with 113 critically ill children, prolonged lymphopenia (lymphocyte count of <1000 cells mm^{-3} for longer than 1 week) was associated with the development of nosocomial infection (OR = 5.5; 95% CI = 1.7–17; $P < 0.05$) and mortality (OR = 6.8; 95% CI = 1.3–34; $P < 0.05$) [23]. Acute-phase proteins, such as CRP, increase after an injury, whereas other proteins decrease, such as serum albumin [5]. Therefore, although the role of CRP is not entirely clear, it is considered as an established biomarker of infection and inflammation, especially in paediatrics [24].

The continued activation of inflammation may lead to prolonged catabolism [1]. It has been established that critically ill children are exposed to a catabolic state, characterised by increased protein turnover and muscle protein breakdown, resulting in a negative protein balance [25]. Lower lean mass and undernutrition, in critically ill children, have been associated with infectious and non-infectious complications, a longer duration of MV, and mortality [26,27]. To promote positive nitrogen balance and lower risk of nutrition deterioration, adequate energy and protein intake should be prioritised. Several nutritional status markers are available for use in critically ill children, such as weight and height and MUAC [4]. However, the adequate measurement of weight and height is a barrier. Immobility, the need for MV, oedema, and haemodynamic instability are the main reasons why weight accuracy is limited in this population [28]. Also, MUAC appears to be less affected by hydration status than weight [29]. Therefore, considering the limitations for weight measurement in a PICU population, our

PICS-ped definition uses MUAC, in addition to albumin, as a surrogate for catabolism.

In the present study, days using vasoactive drugs and antibiotics were associated with PICS-ped. Critically ill children who require antibiotics or vasoactive drugs for a longer period are usually the most severe patients and, consequently, show a higher risk for infection, inflammation, and catabolism. In the first 7 days, energy and protein intake were not associated with PICS-ped. However, it has been shown that cumulative energy deficit, in the first week of PICU admission, is associated with worse clinical outcomes and nutritional status deterioration [4]. Energy and protein delivery are associated with the reduction of infections and mortality rates and with lower PICU LOS [30–32]. Underfeeding during critical illness aggravates the catabolism and may lead to lean body mass reduction in the already undernourished patient [33]. Nonetheless, the excess of energy intake may inhibit autophagy and increase the risk of cell death, organ dysfunction and, consequently, it might be associated with prolonged hospitalisation and mortality [34].

Regardless of the lack of a unified definition, PICS has been associated with poor outcomes in adults. Other than in neonates, [35] no studies were found in the paediatric population. In the present study, patients with PICS-ped had a longer hospital LOS, a longer duration of MV and a higher risk of mortality. PICS in neonates has been associated with metabolic dysregulation, growth impairment and a longer LOS [35]. In a study with 123 adults with enterocutaneous fistula, the incidence of PICS was 43.1%. Moreover, the PICS group experienced longer ICU LOS and a higher rate of mortality (28.3% versus 7.1%) [36]. In the present study, an overall mortality of 10.5% (16/153) was observed. Mortality in patients with PICS-ped was 42.8% (3/7), whereas, in patients without PICS-ped, it was 8.9% (13/146). In a retrospective cohort study conducted with 214 adult patients with severe acute pancreatitis and prolonged intensive care (>14 days), 149 (69.6%) met the criteria of PICS. Patients with PICS showed longer ICU LOS and higher post-ICU mortality (HR = 4.5; 95% CI = 1.2–16.3; $P = 0.024$) [37]. Patients who develop PICS may experience recurrent infectious and inflammatory complications with consequences as readmissions and surgical procedures. Therefore, despite its low incidence, PICS leads to a substantial burden on the patient and on hospitals [38]

In a previous study, at ICU discharge, PICS patients showed significantly worse nutritional status [37]. Furthermore, a substantial loss of lean body mass occurs mainly as a result of the persistent inflammation that leads to catabolism and blocks anabolism, and the decrease in muscle mass appears to happen despite the traditional nutrition support. Also, in the present study, no difference regarding energy and protein prescriptions and goals were found between PICS and no PICS patients. These data may suggest that, in PICS-ped, energy and protein intake were unable to overcome catabolism, and therefore it is important to investigate new nutritional strategies for this subset of critically ill children. In a study conducted with 56 adult patients with CCI, despite receiving nutrition support according to the adult guidelines, sepsis survivors progressed into a chronic malnourished state. The recommendation of nutrition support for patients with CCI and PICS is limited, even for the adult population [39]. Therefore, future studies should investigate anabolic nutrition strategies so that patients progress more quickly to the recovery phase and also provide evidence for the development of protocols to optimise nutrition therapies [1].

The present study has some limitations. Despite being a single-centre study, it was conducted in a reference centre in Southern Brazil. The cut-offs and the parameters proposed for PICS in adults may not be appropriate for the paediatric population and the selected parameters may also be used to describe other diseases' status. However, there is no consensus for the criteria to diagnose PICS, especially in critically ill children. In addition, because MUACz is available for children aged >3 months, we excluded younger children. However, MUAC is considered to be a more feasible measure nutritional status marker than BMI and it appears to be less affected by hydration status than weight, [29] reflecting the nutritional status deterioration more accurately. Also, the median reduction of MUACz was more than 0.67 *Z*-scores, corresponding to nutritional status deterioration [40]. Some of the non-significant differences and unexpected results observed between patients with and without PICS-ped may be a result of the inadequate power and the small sample size. Even though the sample size was small and only 4.58% developed PICS-ped, to our knowledge, this is the first study describing PICS in a pediatric population. Considering the sample size, and the wide 95% CI, we suggest that future studies validate and confirm the definition of PICS-ped in critically ill children.

Conclusions

In an exploratory study of PICU patients, PICS-ped exists in critically ill children. Although it was observed in only 4.6% of the patients, PICS-ped should be investigated in futures studies. The PICS-ped for critically ill children defined in the present study was associated with duration of MV, hospital LOS and mortality, even in a subset of critically ill children mechanically ventilated for more than 48 h. Efforts to determine the factors associated with PICS-ped are important for improving outcomes in this population.

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Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.
DBH, LDAO and JCV received a scholarship provided by the Coordination for the Improvement of Higher Education Personnel (CAPES).
DBH, LDAO and JCV conceived and designed the study, designed the data collection instruments, collected data, carried out the analyses, contributed to the interpretation of the data, and drafted the initial manuscript. MSF, EB and NLB contributed to the design of the research and the interpretation of the data, and drafted the initial manuscript. YMFM conceived and designed the study, designed the data collection instruments, coordinated and supervised data collection, carried out the analyses, contributed to the interpretation of the data, and drafted the initial manuscript. All authors have critically reviewed its content, approved the final manuscript submitted for publication and agree to be accountable for all aspects of the work.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with STROBE. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Recruitment flowchart.

OLDER ADULTS

An investigation of recommended serve food portions and attaining energy and protein requirements in older adults living in residential care

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Keywords
aged care, serve size, menu, energy, protein, malnutrition.

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Abstract

Background: Ageing populations show a propensity for reduced food intake, which impacts nutritional adequacy. Nutrition guidelines for residential care homes (RCHs) are currently based on serve size of core food groups and do not consider nutrient density. The present study aimed to investigate the weight of foods served/consumed compared to recommended serve sizes and to compare energy and protein intake with individual requirements.
Methods: This was an observational study of older adults living in four RCHs. Dietary intake was estimated through the difference between weighed reference meals and a single, double-weighed 24-h food plate waste collected from each participant. FoodWorks9® (Xyris® Software, Brisbane, Australia) was used to calculate energy, protein and serves of core food groups from food intake and the menu provided to recommended serve sizes. Individual intake was compared with nutrition guidelines and estimated energy and protein requirements.
Results: Across 420 participants, 9.8% completed a main meal (lunch or dinner). The servings provided [248 g; interquartile range (IQR) = 206–290 g] were less than the recommended servings for a main meal (306 g = protein/starch/two vegetables), with 157 g (IQR = 109–221 g) consumed. The menu provided for minimum serves of all core food groups except for dairy. Median energy intake (n = 389) (5272 kJ day^{-1}, IQR = 4229–6720 kJ) and protein intake (47.3 g day^{-1}, IQR = 35.9–60.8 g) were less than estimated requirements (8181 kJ day^{-1}, IQR = 7300–9338 kJ day^{-1}; 76.7 g day^{-1}, IQR = 66.7–90.8 g).
Conclusions: Nutritional needs were not met in this cohort. The findings of the present study highlight the need for smaller, nutrient-dense meals and revised menu standards to ensure nutritional adequacy in this vulnerable population.

Introduction

Given the high rates of undernutrition in aged care older adults and the nutritional dependence on aged care homes to meet dietary needs, the menu and food supply are critical to optimising overall health [(1)]. The prevalence of 20% malnutrition in older adults living in residential care homes (RCHs) is variable largely as a result of the measurement tools used and the variety of definitions [(2)]; however, inadequate nutrition leads to unintentional weight loss and an increased risk of morbidity and mortality [(2–5)]. Reduced appetite associated with peripheral and sensory ageing, ageing of the gut, social and environmental factors, variable care, and dining models all

contribute to this decline [6,7]. These factors, combined with other nutrition-related problems, such as dementia, diabetes, dysphagia and frailty, add to the difficulties in meeting nutritional needs of older adults living in RCHs. The availability of non-specific population menu guidelines and serve size recommendations add to the challenges of meeting the energy and protein needs of this vulnerable group.

In Australia, there are no national guidelines for RCH menus; rather, there are state-based guidelines for hospitals and aged care that are based on population guidelines, not specific to aged care requirements. The myriad of dietary requirements for medical conditions requires menus to be flexible with nutrient-dense options and variety [8,9]. Even a well-planned menu following a variety of food groups and accredited standards for menu planning can still result in iatrogenic malnutrition [10,11].

Previous research has suggested that portion sizes are too large for older adults with reduced appetites [4,12–15]. Even if the menu is considered nutritionally adequate, older adults may still be unable to consume their daily nutritional requirements [16,17]. A consistent intake of greater than 75% of a meal may reduce malnutrition risk [2,18]; hence, further research and specific guidelines for menu and recipe development are warranted. An understanding of serve sizes can inform the development of such guidelines specific to the dietary needs of older adults residing in RCHs [10].

Meeting nutritional needs, dietary preferences and reduced food intake of older adults within set guidelines and budget is challenging [19,20]. A range of strategies, including the implementation of high energy mid-meals and snacks, oral nutritional support (ONS) products and fortified foods, have been evaluated previously [21–23]. The problem of malnutrition in this population will persist without examining the root causes of insufficient energy and protein intake and implementing feasible solutions.

The present study aimed to investigate the weight of foods served/consumed compared to recommended serve sizes and to compare energy and protein intake with individual requirements.

Materials and methods

Study design and setting

A multi-centre observational study was conducted in Melbourne, Australia in October 2018, with four participating RCHs. The RCHs were based in a metropolitan area, privately owned with some government funding, comprising a variety of cultural backgrounds and care needs, including higher complex care and specific dementia care areas. The RCHs maintained their dining environments and foodservice models during the observational period, and meals in all facilities were provided by a well-established contract catering company. The cook-fresh on-site foodservice models and dining experiences included tray delivery to rooms, small 'family-style' dining rooms served from kitchenettes, and large dining rooms served from the main kitchen or satellite serveries. Meal service at each RCH was at approximately 08.00 h for breakfast, mid-day or 12.30 h for lunch, and 17.00 h for dinner; mid-meals were provided between these periods. Lunch and dinner main menu choice was considered the main meal (excluding soup and dessert).

Participants

Participants were older adults who consumed all meals on-site on the day of observation and were consuming a Regular and Easy to Chew diet texture (Level 7), in accordance with the International Dysphagia Diet Standardisation Initiative (IDDSI) Framework [24]. Older adults excluded from the study were those requiring a modified texture diet (Level 6 soft and bite-sized, Level 5 minced and moist, Level 4 puree and Level 3 liquidised), those who ate food off-site during the observation day, had illness or a medical condition that would greatly affect intake (i.e. influenza/ gastroenteritis), or who were receiving enteral tube feeding or end-of-life care.

Data collection

Following intensive training and supervision by the principal researcher, final year nutrition and dietetic students collected observational data over 15 days. Data were collected once for each participant, over 24 h from Monday to Friday for breakfast, morning tea, lunch, afternoon tea and dinner. Where plates were removed prior to being weighed, data were excluded from nutrition analysis.

Assessment of food intake

Reference meals in three sizes (small, medium, large) were plated and weighed to use as a guide for estimating plate waste. All food weights were recorded in duplicate on calibrated digital scales (HC6000, ZL: 2006300144733 CWScales, Changzho, China; maximum capacity: 6000 g, resolution 1.0 g) after accounting for plate weight. Food intake of participants was estimated as the difference between the weight of the reference meals and the amount of weighed food waste [25].

Nutritional analysis was conducted using FoodWorks®9 (Xyris® Software, Brisbane, QLD, Australia). In the case of unknown brands, the most appropriate ingredients were selected from AUSNUT2013, AusBrands 2017 and AusFoods 2017 (Foodworks®9). Nutrition information panels were used where product brands were specified.

Estimating energy and protein intake

Energy and protein intake were estimated from the 24-h food intake data for each participant for the three mealtimes (breakfast, main meals, including soup and dessert) and mid-meals. Intake from drinks and ONS products was not observed as part of the present study because their consumption was not directly linked to the primary aim of determining serve sizes of meals and mid-meals.

An assumption was made that all drinks provided were consumed in their entirety (980 kJ and 5.3 g of protein per resident per day) and added to the total energy and protein intake calculations. ONS products and prescribed high protein milkshakes were excluded because they were not considered part of the standard menu.

Energy and protein requirements of participants were estimated as follows: protein requirements were calculated using 1.2 g kg body weight^{-1} [26]; energy requirements were based on the Mifflin–St Jeor equation for males and females [27]; a physical activity level (PAL) of 1.3 was applied [28].

Comparison of intake against core food groups

Data estimating participant dietary intake across the core food groups using the Australian Guide to Healthy Eating (AGHE) were provided within FoodWorks9®. Discretionary foods (e.g. cakes, biscuits, some fats) are part of the AGHE and were added manually where information was missing from the database.

Demographic data

Participant gender, age, height, weight, meal assistance, whether they had ONS products prescribed, and diagnoses (i.e. diabetes, respiratory heart disease, neurodegeneration, gastrointestinal, bone health and wounds) were extracted from the clinical notes. Height was estimated using ulnar length [29] if it was not documented elsewhere. Body mass index (BMI) was calculated using the equation weight (kg)/height (m)^2. Energy intake was categorised into the following BMI ranges for the aged population: BMI <23 (higher risk of mortality), BMI 23.1–33 (healthy weight range), and BMI >33.1 (higher risk of mortality) [30]. BMI data were also compared against the ESPEN diagnostic criteria for malnutrition [26]. Meal assistance (e.g. no assistance, partial physical assistance or full physical assistance) information was gathered from the resident care plan and corroborated with observation at the mealtime.

Ethical approval

Ethical approval was granted by Monash University Human Research Ethics Committee (Project 13770). In accordance with the ethical approval, all older adults were included in the study, unless they opted out. An explanatory statement and poster were distributed by RCH managers informed older adults and their family members. The study has been reported in accordance with the STROBE guidelines [31].

Statistical analysis

Statistical analysis was undertaken using SPSS, version 25 (IBM Corp. Armonk, NY, USA). Non-parametric descriptive statistics were performed to describe demographics and characteristics. All variables presented a skewed distribution with the results expressed as the median and interquartile range (IQR). Food intake and intake of core food groups were analysed using FoodWorks®9 and compared with the AGHE [32]. Spearman's rank-order correlation (rho) was used to compare relationships of energy and protein intake with BMI. Mann–Whitney *U*-tests were used to compare gender, BMI, diagnosis and the use of ONS products with energy intake. Chi-squared tests for independence for significant associations between main meal consumption and gender, meal assistance and dementia, and dementia and BMI. A Jonckheere–Terpstra test and Kendall tau-b were used to review trends of intake across the meal service (dining room and tray service). Energy and protein intake required a full 24-h data set. Meal waste weight included all meals collected. $P < 0.05$ was considered statistically significant.

Results

In total, 420 older adults participated in the present study. Participant characteristics are presented in Table 1. A large number of participants had cardiovascular disease, whereas 60.2% had a diagnosis of dementia. The median age was 86 years (IQR = 80–91), median BMI was 25.3 kg m^{-2}, 67.6% were female and almost 70% were independent eaters. The weight of meals consumed was obtained for 420 older adults and there were 389 older adults who had complete 24-h intake for energy and protein calculations. There were no statistical differences in energy and protein intake with gender, differential diagnosis of dementia, or being prescribed an ONS product. Only 10.7% ($n = 45$) of the participants had a prescribed ONS product.

Weight of foods served and consumed

Breakfast consisted of cereal/porridge and bread/toast, a median weight of 236 g (IQR = 178–239 g) for cereal and milk and 182 g (IQR = 148–240 g) porridge was served compared to 184 g (IQR = 125–236 g) and 148 g

Table 1 Demographic data

Participants (n = 420)	
Age (years), median (IQR)	86 (80;91)
Males, n (%)	136 (32.4)
Females, n (%)	284 (67.6)
Residential care home (n = 420 of 574 beds; 73%)	
RCH 1, n	129
RCH 2, n	145
RCH 3, n	65
RCH 4, n	81
Clinical indicators* (n = 419)	
Weight (kg), median (IQR)	68.1 (57.6–80.2)
Female weight (kg), median (IQR)	62.2 (54.2–74.8)
Male weight (kg), median (IQR)	77.2 (69.4–87.7)
Height (m), median (IQR)	1.64 (1.57–1.68)
Female height (m), median (IQR)	1.60 (1.55–1.65)
Male height (m), median (IQR)	1.71 (1.66–1.78)
BMI (kg m^{-2}), median (IQR)	25.3 (22.0–29.6)
BMI ≤23, n (%)	131 (31.3)
BMI 23.1–33, n (%)	237 (56.6)
BMI ≥33.1, n (%)	51 (12.2)
Diagnosis* (n = 420) (%)	
Diabetes mellitus (n, %)	105 (25.0)
Cardiovascular disease (n, %)	315 (75.0)
Respiratory disease (n, %)	25 (6.0)
Dementia (n, %)	253 (60.2)
Other neurodegeneration (n, %)	26 (6.2)
Upper gastrointestinal disease (n, %)	43 (10.2)
Lower gastrointestinal disease (n, %)	14 (3.3)
Renal disease (n, %)	21 (5.0)
Bone health (n, %) (osteoporosis/osteopenia/osteoarthritis)	207 (49.3)
Falls/fracture history (n, %)	174 (41.4)
Evidence of wound/pressure injury (n, %)	21 (5.0)
Meal assistance, n = 420 (%)	
Independent (n, %)	290 (69)
Partial assistance (n, %)	78 (18.6)
Full assistance (n, %)	52 (12.4)
Oral nutritional support product (n = 420) (%)	
No oral nutritional support product (n, %)	375 (89.3)
Ordered oral nutritional support product (n, %)	45 (10.7)

BMI, body mass index (Winter *et al.* 2014); RCH, residential care home. Meal assistance, independent required no assistance with meals, partial assistance required physical or verbal assistance during the meal, and full assistance required full assistance for the duration of the meal.

*Participants could have more than one diagnosis so aggregate exceeds 100%.

(IQR = 115–203 g) consumed, respectively. The median weight of bread/toast served was 75 g (IQR = 62–82 g) and 54 g (IQR = 35–75 g) was consumed. Lunch and dinner main meals (protein/starch/vegetables) weighed 242 g (IQR = 195–301 g) and 249 g (IQR = 192–298 g), respectively; however, only 166 g (IQR = 95–236 g) was consumed at lunch and 143 g (IQR = 80–230 g) at dinner. Overall, median main meal (lunch and dinner) weight served was 248 g (IQR = 206–290 g), although a median of 157 g (IQR = 109–221 g) was consumed. Only 40 (9.8%) older adults ate 100% of the main meal.

The median weight of the protein at the main meal (e.g. meat/poultry/fish/egg) served was 110 g (IQR = 83–129 g), vegetables 43 g (IQR = 35–58 g) and potato/grain 111 g (IQR = 69–142 g); however, 69 g (IQR = 40–94 g) of main protein, 31 g (IQR = 16–48 g) of vegetables and 68 g (IQR = 35–112 g) of potato/grain were consumed. The median weight of desserts offered was 96 g (IQR = 68–121 g) with 67 g (IQR = 44–98 g) consumed. The median snack served was 45 g (IQR = 31–64 g) and 38 g (IQR = 38–60 g) was consumed. Details of the weight (gram) of meal items served and consumed are provided in the Supporting information (Table S1).

Of the mean weight of portions served and consumed, the percentage consumption was 88% of breakfast, 62% of the protein portion of the meal, 73% of vegetables, 68% of potato/grains, 71% of dessert and 88% of snacks.

Core food groups

Table 2 shows the core food groups for the 15 days of the provided menu, as well as the number of serves consumed, across all four RCHs, in comparison with the AGHE serve size guidelines. All core food groups for protein, grains, vegetables and fruit, except dairy, were provided by the menu. Even when the weight of foods provided was the recommended serve size, the amount consumed was less than provided; for example, a median of 81 g (IQR = 52–124 g) of rice was served, and 35 g (IQR = 17–81) of rice was consumed, compared to the recommended AGHE serve of 75–120 g to provide one serve of grains. Additional foods (i.e. discretionary foods) contributed 1.3 serves to the daily intake.

Vegetable intake was less than the five recommended serves per day (consumed a median 2.9 vegetable core food group). Soup provided a median vegetable serve of 55.5 g (IQR = 43.7–75.9 g) or 0.7 (IQR = 0.6–1.0) core vegetable food group serves. A median of 0.4 serves of green vegetables, 0.5 serves of orange vegetables, 0.3 serves of white vegetables and 1.0 serves of potato were consumed daily.

Energy and protein intake compared to requirements

Table 3 presents the energy and protein of food served and consumed compared to the estimated requirements across all participants. Details of intakes according to meal service (dining room or tray) are provided in the Supporting information (Table S2). Although median energy requirements were 6215 kJ day^{-1} (n = 419, IQR = 5370–7627 kJ), older adults were only able to consume 5272 kJ

Table 2 Core food groups of food served and consumed over 15 days on menu cycle over four residential care homes compared with Australian Guide to Healthy Eating (AGHE) Food Groups

	Protein serves	Grain serves	Vegetable serves	Fruit serves	Dairy serves
Core food groups provided and consumed					
Serves per day provided on menu*					
Median	2.3	5.2	5.5	1.5	1.0
IQR	2.3–2.4	5.1–5.7	5.3–5.7	1.4–1.6	1.0–1.0
Menu modelling drinks served†				1.2	0.4
Serves per day consumed‡ (n = 400)					
Median	1.0	3.7	2.9	0.5	0.8
IQR	0.6–1.6	2.7–5.5	1.8–4.1	0.2–1	0.4–1
'Extras/discretionary' (n, grams)		260, 34			200, 70
IQR		(23–53)			(45–133)
'Extras/discretionary' (n, serves)		260, 1			200, 0.3
IQR		(0.9–1)			(0.2–0.5)
Percent of served core food groups consumed compared to served	43%	90%	53%	18%	79%
AGHE Guidelines					
AGHE core food group serves per day	2.5 (men)	4.5 (men)	5 (men)	2 (men)	3.5 (men)
	2 (women)	3 (women)	5 (women)	2 (women)	4 (women)
Percentage consumed (from served portion)	62% protein	55% rice 96% other grain	69% potato 73% vegetable		

*Serves based on food provided over 15-day menu cycle for four menus, no other fluids such as milk drinks and fruit juices included, based on the first choice option main meal.

†Drinks (tea, coffee, juice, cordial) offered based on 150 mL at meals and snacks.

‡Food groups calculated using FoodWorks9 do not incorporate 'extras/discretionary'. Extras/discretionary foods include all biscuits, muffin, scone and cakes consumed. AGHE, Australian Guide to Healthy Eating (2013). FoodWorks9 was used to determine grams for serves for examples of AGHE serves when a piece of fruit or cup was measured.

day^{-1} (IQR = 4229–6720 kJ) despite the menu providing 8181 kJ day^{-1} (IQR = 7300–9338 kJ). Median protein requirements (n = 389) were 81.7 g (IQR = 65.5–96.2) per day; older adults consumed 47.3 g (IQR = 35.9–60.6 g), whereas 76.7 g (IQR 66.7–90.8) was provided on the menu. Protein intake was distributed across the day as follows: 15 g of protein at breakfast, 29 g at lunch and 29 g at dinner. Median protein intake was 11 g at breakfast, 18 g at lunch and 14 g at dinner.

Body mass index

There was a positive correlation observed between BMI category [30] and energy intake (r = −0.164, n = 388, P = 0.001). Increases in energy intake were associated with increasing BMI categories (BMI ≤23, n = 117; BMI 23.1–33, n = 223; BMI ≥33.1, n = 48; χ^2 = 5.86, d.f = 2, n = 388, P = 0.05). There was no difference in protein intake across the BMI categories (Table 3; see also Supporting information, Fig. S1). When compared against the ESPEN BMI criteria for malnutrition, 7.6% of the older adults had a BMI ≤18.5; 17.4% had a BMI in the range 18.6–22; 62.8% had a BMI in the range 22.1–32; and 12.2% had a BMI ≥32.1 [26].

Discussion

The present study aimed to investigate the weight of foods served/consumed compared to recommended serve sizes, as well as to compare energy and protein intake with individual requirements in RCHs. We observed that (i) serve sizes provided were smaller than recommendations and (ii) 90.2% of participants were unable to consume the full meal that they received. The menu provided sufficient energy and protein if all menu items were consumed; however, actual intake fell short of requirements.

This research aligns with recent professional consensus documents that define future research priorities. Both in the UK [33] and in Australia [34], research that extends the understanding of identification and management of malnutrition in older populations is a priority for dietetic research. Internationally, researchers have found similar problems with food guides underpinning RCH menus and reiterated the need for guidelines for menu development for the aged population rather than using a food guide aimed for a healthy older population [14,35]. In the present study, we have shown that menus based on population nutrition guidelines do not meet the nutritional

Table 3 Energy and protein of food served and consumed compared with estimated requirements in four residential care homes

Description	Males	Females	All
Energy			
Estimated energy served from menu [n = 389, kJ (IQR)]			8181 (7300–9338)
Estimated energy requirements [kJ day^{-1} (n, IQR)]	7866 (n = 135, 7363–8550)	5640 (n = 284, 5053–6454)	6215* (n = 389, 5370–7627)
Total energy consumed [kJ (n, IQR)]	5578 (n = 135, 4246–6703)	5166 (n = 284, 4261–6721)	5272 (n = 389, 4229–6720)
Energy deficit between requirements and consumption [kJ (n, IQR)][§]	2288 (n = 135, 1163–3620)	474 (n = 284, 1081–1379)	843 (n = 389, 505–1986)
Energy deficit between served and consumped [kJ (n, IQR)]**	2603 (n = 135, 1478–3935)	3015 (n = 284, 1460–3920)	2909 (n = 284, 1461–3952)
BMI ≤ 23[‡] [kJ (IQR)], P = 0.003			4874 (4030–5952)
BMI 23.1–33[‡] [kJ (IQR)], P = 0.003			5542 (4325–6931)
BMI ≥33.1[§] [kJ (IQR)], P = 0.003			5787 (4538–7261)
Protein			
Estimated protein served (n = 389) [g (IQR)]			76.7 (66.7–90.8)
Estimated protein requirements [g day^{-1} (n, IQR)]	92.6 (n = 135, 83.3–105.2)	74.6 (n = 284, 65.0–89.8)	81.7[†] (n = 389, 65.6–96.2)
Total protein consumed (g (n, IQR))	48.1 (n = 135, 37.7–63.5)	46.7 (n = 284, 35.3–58.9)	47.3 (n = 389, 35.9–60.6)
Protein deficit between requirements and consumption [g (n, IQR)][§]	44.5 (n = 135, 29.1–54.9)	27.9 (n = 284, 15.7–39.3)	34.4 (n = 389, 21.1–45.8)
Protein deficit between served and consumed [g (n, IQR)]**	28.6 (n = 135, 13.2–47.6)	30 (n = 284, 15.7–39.3)	29.4 (n = 389, 16.1–40.8)
BMI ≤23[‡] [g protein (n, IQR)], P = 0.007			43 (n = 117, 33–55)
BMI 23.1–33[‡] [g protein (n, IQR)], P = 0.007			49 (n = 223, 38–64)
BMI ≥33.1[‡] [g protein (n, IQR)], P = 0.007			48 (n = 48, 36–71)

BMI, body mass index; IQR, interquartile range.

*Mifflin–St Joer equation × 1.3 physical activity level (PAL).

[†]1.2 g protein kg body weight^{-1}.

[‡]Post-hoc tests were performed with Bonferroni correction. BMI ≤23 and BMI 23.1–33 (P = 0.011) and BMI ≤23 and BMI ≥33.1 (P = 0.014).

[§]Difference in energy and protein served, consumed and required, modelled intake.

**Difference in energy and protein served, requirements, and consumed, modelled intake.

requirements of older adults in RCHs. We also identified that older adults in RCHs are unable to manage the current serve sizes provided. As such, a review of the serve size recommendations that underpin RCH menus is warranted.

Menu development is a complex task with the literature purporting portion sizes playing a small but crucial role [(36)]. The challenges of menu development based on a food guide's portion size and the number of serves are well documented [(14)], with complications associated with ageing such as poor appetite affecting overall intake. Our study showed that 63.3% of the offered main meals were consumed, which is lower than the target of a consistent intake of 75% of meals [(2,5,18,37)]; this suggests that the recommended serve sizes are too large for older adults in aged care.

Recommendations for portion sizes are described in units of weight. Irrespective of the menu provided in the RCHs in the present study, older adults were unable to consume the weight of food provided. A similar study in a community setting, in the UK, where aged care older adults consumed 301 g (protein/starch/vegetable), was lower than the recommended serves sizes [(38)], yet higher than those reported in the present study. Our study population in the RCH setting ate 144 g less than this community population. The current recommended serves for a main meal using the AGHE is 306 g (protein, potato and two vegetables) [(32)]. Therefore, the current AGHE recommended serves for the main meal appear more suited to the needs of a community setting aged population rather than the frail older population.

Many RCH menus fail to meet resident nutritional needs [(35,39–41)]. Improvement strategies in Canada for long-term care menus have been successful, whereby the introduction of nutrient-dense meals and thoughtful use of ingredients met the food guide, protein and energy requirements for an insignificant additional cost [(40)]. A Meals on Wheels study in Australia showed that, by increasing the energy density of meals, serving size was reduced to less than the current recommendations without compromising nutritional quality [(42,43)]. These two studies demonstrate that nutritional requirements may be met with attention to ingredient and recipe formulation,

rather than only establishing nutrition adequacy based on core food groups. Regardless of meeting core food group requirements (except for dairy) in the present study, older adults were unable to achieve the recommended consumption of these core food groups and, subsequently, energy and protein intake.

Energy intakes amongst older adults living in aged care have been widely studied. A standard menu can provide between 5682 and 8643 kJ day^{-1} [44,45]. Energy provided on menus should be optimised; the waste factor should be calculated and considered because oral intake can vary from 15% to 77% [5]. The present study showed an inadequate estimated energy intake compared to recent studies in aged populations [45–47]. In one study, where portions of food were similar to that of the present study, energy and protein requirements were achieved, possibly as a result of the cultural cuisine being higher in fat and protein-heavy foods. Nutrient-dense recipes and their fortification is therefore a viable option for achieving nutritional adequacy with smaller serves [12,44,48–51].

Tieland *et al.* [52] investigated the distribution of protein intake over the day. Those who spread the protein across the day had overall higher intakes despite the relative protein intake being inadequate [52]. The current recommendation is at least 25 g protein in a meal [52]. In the present study, despite adequate protein provided in the menu, the menu had a lower protein provision at breakfast compared to lunch and dinner, and very little protein at snacks. Consumption of the protein serve was also smaller than served protein at lunch and dinner. In practice, including protein, at each meal and snack, possibly with protein fortification, maybe a useful strategy for ensuring an adequate intake of these participants.

Poor food intake and low BMI are a strong predictor of mortality in the elderly population [30,53,54]. Although the present study did not explore mortality rates, the older adults in the BMI range of <23 kg m^{-2} had low energy intakes similar to studies showing the inverse relationship of energy and mortality rate. [54]. Interestingly, there was no difference in protein intake and BMI category. From the present study, the provision and intake of energy and protein on the menu of the older adult population must be developed for those across all BMI ranges with an emphasis on increased ways to assist with meeting protein needs.

Although intake can vary as a result of medical, environmental and social factors, menu development needs to take into account smaller appetites to achieve nutritional adequacy, including for older adults with dementia. In addition to nutritious menus, other strategies are required for improved intake through the meal experience, such as meal assistance, a person-centred care approach, meal delivery style, environmental considerations, routine and social interaction, and family-style dining [44,55–59]. Researchers have not reported robust evidence that environmental and behavioural modification improve oral intake in people with dementia [60]; however, multicomponent strategies assist with improving intake [61]. Improvement of overall intake should also address inadequate micronutrient intake [19]. The present study showed an increased intake of energy, protein (both statistically significant) and amount of food (grams, not statistically significant) in those family-style small dining areas, as well as with the type of meal service delivery. Although the meal experience may have had some influence on intake, achieving a nutritionally dense menu, in both micro and macronutrients, as well as careful menu planning, is needed [44,62].

The present study is novel because, to our knowledge, it is the first to review a menu's core food groups and serve sizes with respect to intake in Australian RCHs. This finding can be useful internationally for menu development and provides greater knowledge to the dietetic profession of the small portions consumed by older adults in RCHs. Several limitations are acknowledged. Although 24-h intake was collected for each resident, rather than a 3-day food intake, the consumption of a wide variety of meals and snacks over a 15-day period was observed to ensure that the weights of meals and snacks were representative. Meals in the four RCHs studied were prepared by one catering group where taste, an expectation of meals and presentation may have influenced food intake; however, they did provide consistency of recipe and food provision. Cultural diversity with cultural diet preferences was not investigated, which may have influenced the intake of the menu presented. Fluids were not directly observed but rather were modelled for the estimates of energy and protein intake. The meal dining service and assistance level may have affected intake. Also, the actual intake of ONS products was not recorded; therefore, the deficits modelled in the present study only reflect intake from the menu and no other sources. The potential effect of ONS products with respect to influencing mealtime consumption is acknowledged.

Conclusions

The present study has shown that serve sizes are too large, and it is unachievable for older adults of RCHs to meet their nutritional requirements despite a menu providing adequate nutrition. The study highlights the need for further research for smaller, nutrient-dense meals, revised standards for menu and recipe development, and further exploration into the meal experience for improved energy and protein intake. Furthermore, standards and guidelines specific to older people and frail older adults residing in

RCHs should be developed to enable the delivery of evidence-based nutrition care.

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Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.
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All authors contributed to the conception and design of this research. LS supervised data collection, analysed the data and drafted the manuscript. JP and MB co-supervised data collection and critically reviewed the manuscript. All authors approved the final version submitted for publication.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study reported. The reporting of this work is compliant with the STROBE guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. BMI and energy intake, $n = 388$, $P = 0.003$, Kruskal–Wallis test with significance adjusted by Bonferroni correction of multiple tests. BMI ≤23.0 to 23.1–33.0, $P = 0.010$; BMI ≤23.0 to 33.1+, $P = 0.014$; BMI 23.1 to 33.1+, $P = 1.000$.

Table S1. Weight of meal in grams served and consumed in categories.

Table S2. Energy intake with meal service and experience.

Table S3. Detailed demographics for each residential care home (RCH).

OLDER ADULTS

A higher protein intake at breakfast and lunch is associated with a higher total daily protein intake in older adults: a post-hoc cross-sectional analysis of four randomised controlled trials

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breakfast, dietary protein intake, lunch, older adults, sarcopenia, satiety.

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Abstract

Background: A protein intake of 30-40 g per meal is suggested to maximally stimulate muscle protein synthesis in older adults and could therefore contribute to the prevention of sarcopenia. Protein intake at breakfast and lunch is often low and offers a great opportunity to improve daily protein intake. Protein, however, is known for its satiating effects. Therefore, we explored the association between the amount of protein intake at breakfast and lunch and total daily protein intake in older adults.
Methods: Protein intake was assessed by a 3-day food record in 498 community dwelling older adults (≥55 years) participating different lifestyle interventions. Linear mixed model analysis was used to examine the association between protein intake at breakfast or lunch and total daily protein intake, adjusted for sex, age, body mass index, smoking status, study and total energy intake.
Results: After adjustment for potential confounders, a 10 g higher protein intake at breakfast was associated with a 3.2 g higher total daily protein intake ($P = 0.008$) for males and a 4.9 g ($P < 0.001$) higher total daily protein intake for females. A 10 g higher protein intake at lunch was associated with a 3.7 g higher total daily protein intake ($P < 0.001$) for males, and a 5.8 g higher total daily protein intake ($P < 0.001$) for females.
Conclusions: A higher protein intake at breakfast and lunch is associated with a higher total daily protein intake in community dwelling older adults. Stimulating a higher protein intake at breakfast and lunch might represent a promising nutritional strategy to optimise the amount of protein per meal without compromising total daily protein intake.

Introduction

Our society is ageing rapidly [(1)]. Ageing is associated with loss of muscle mass, strength and performance, a process termed sarcopenia. Sarcopenia is associated with an increased risk of falls and fractures, morbidity and mortality. To prevent or even counteract sarcopenia is of major importance because it declines the risk for adverse health

outcomes and health-related cost and improves quality of life [2]. The cause of sarcopenia is multifactorial and includes physical inactivity and lower protein intakes [3]. Increasing dietary protein intake has been suggested as important beneficial strategy for preventing and/or treat sarcopenia in older adults [4,5].

Phillips *et al.* [6] suggested that a dietary protein intake per meal of 0.4 – 0.6 g kg body weight^{-1} (BW) or approximately 30-40 g is necessary to maximally stimulate skeletal muscle protein synthesis in older adults. Most community dwelling older adults in the Netherlands do not reach these suggested amounts of protein per meal, particularly at breakfast and lunch: mean (SD) protein intake is 11 ± 7 g at breakfast and 18 ± 10 g at lunch [7]. Multiple researchers suggest that an even distribution of proteins over the three meals (and therefore higher protein intakes at breakfast and lunch) with sufficient amounts of protein per meal could translate into a higher anabolic response [8-11]. Kim *et al.* [10] concluded that probably the most efficient way of maximising the anabolic response is to increase dietary protein intake at breakfast and lunch, without reducing protein intake at dinner (for consumption patterns with the hot meal in the evening). Because protein intake at breakfast and lunch in older adults is low [7], these meals offer great potential to increase daily protein intakes [12], aiming to stimulate muscle protein synthesis and optimise muscle maintenance [13,14].

Proteins, however, have a strong satiating effect [15]. Increasing the intake in one meal may result in a compensation of protein intakes and other nutrients and energy at other meals [16]. This compensation may be influenced by ageing because ageing affects hunger and satiety hormone secretion, as well as feelings of hunger and fullness [17]. However, the relationship between protein at breakfast or lunch and total daily protein intake in older adults is unclear [18,19]. Therefore, the present study aimed to explore the association between the amount of protein intake at breakfast and at lunch and total daily protein intake in community dwelling older adults.

Materials and methods

Study design and study population

A cross-sectional analysis was performed on baseline data of older adults (≥55 years) participating one of four different lifestyle interventions in the Amsterdam Nutritional Assessment Center at Amsterdam University of Applied Sciences. The four lifestyle interventions were:

1 The MPS (Muscle Preservation Study) [20]: a randomised controlled trial in which the effect of a high whey protein-, leucine- and vitamin D-enriched supplement was tested during a 13-week weight loss programme including resistance exercise on preservation of muscle mass in an older (≥ 55 years) obese adults. Obesity was defined as a body mass index (BMI) ≥ 30 kg m^{-2} or as a BMI ≥ 28 kg m^{-2} with waist circumference > 88 cm (women) or > 102 cm (men).
2 The WelPrex (Weight Loss with Protein and Exercise) study [21]: a randomised controlled trial in which the effect of a high protein diet and/or three times per week resistance exercise was tested during a 10-week weight loss programme in older (≥ 55 years) overweight and obese adults. Overweight was defined as a BMI ≥ 28 or as a BMI > 25 kg m^{-2} with waist circumference > 88 cm (women) or > 102 cm (men).
3 The PROBE (protein and lifestyle intervention to preserve muscle mass in obese older type 2 diabetes patients) study [22]: a randomised controlled trial comparable to the MPS, a 13-week weight loss trial including resistance training in which the effect of the same supplement was tested, although this population was a diabetic older (≥55 years and older) population with obesity. Obesity was defined as a BMI ≥ 30 kg m^{-2} or as a BMI ≥ 27 kg m^{-2} with waist circumference > 88 cm (women) or > 102 cm (men).
4 The VITAMIN (VITal AMsterdam older adults IN the city) study [23]: a randomised controlled trial that evaluated the effectiveness of a digitally supported home-based exercise training programme, as well as the additional value of dietary protein on physical performance, in community dwelling older adults aged ≥ 55 years.

A full description of the eligibility criteria is available online in the Dutch Trial Register (MPS: NL2623; WelPrex: NL4434; PROBE: NL4357; VITAMINE: NL5472; http://www.trialregister.nl). Written informed consent was obtained from all subjects and the studies were performed in accordance with the Declaration of Helsinki. These studies took place from March 2011 to September 2018 in the Amsterdam Nutritional Assessment Center at the Amsterdam University of Applied Sciences, Amsterdam, The Netherlands.

Assessment of dietary intake

Baseline dietary intake was assessed by a 3-day food record at 2 week days and 1 weekend day. Food records were prestructured for the following eating moments: breakfast, in between breakfast and lunch, lunch, in between lunch and dinner, dinner, and in the evening. Subjects were asked to report their food intake as specific as possible and to report amounts of their intake in standard household measures (e.g. three slices of whole grain bread) or to weigh their food items on a kitchen weighing

scale. Food records were checked for completeness during study visits by trained fourth grade students Nutrition and Dietetics under supervision of the study dietician. Additional information about unclear items or amounts was obtained and recorded. Food record data of the four studies were collected and verified in accordance with the standard operating procedures of our laboratory. The food items were coded and the nutritional intake data file was coupled to the computerised Dutch Food Composition Table [24,25] to calculate total energy and macronutrient intakes. The dietician or coordinating investigator performed an additional verification and consistency check after the coding process. Subjects with completed dietary records on at least 2 days, and with average reported energy intake of at least 800 kcal day^{-1} were included for analysis. The outcome variable total daily protein intake was calculated in g, g kg BW^{-1} and g kg fat free mass $(FFM)^{-1}$. Protein intake in g kg BW^{-1} was also adjusted for body weight for subjects with a BMI $\geq$ 30 kg m^{-2} using body weight at BMI 27.5 kg m^{-2} [26] and for subjects with a BMI < 22 kg m^{-2} using body weight at BMI 22 kg m^{-2} [27]. This adjustment of body weight is applied to make it more comparable to true protein needs and to make it more comparable to that often used in dietetic practice because body composition parameters are not always available. FFM in obese subjects is low relative to their body weight and therefore using actual body weight would probably overestimate protein needs. The opposite is the case for subjects with a low BMI: then, FFM is relatively high for their body weight, and using actual body weight would probably underestimate true needs.

Assessment of general characteristics and potential confounders

Body composition, including fat mass (FM) and FFM, was determined using air displacement plethysmography (BODPOD, Life Measurement Inc., Concord, CA, USA). Body weight was measured on the calibrated scale as part of the BODPOD system. Body height was measured to the nearest 0.5 cm using a wall-mounted stadiometer (Seca 222; Seca, Hamburg, Germany). Waist circumference was measured in a standing position halfway between the anterior superior iliac spine and the lower rib after normal expiration (Seca 201; Seca). General characteristics (gender, age and smoking status (current smoker yes or no) were self-reported at baseline.

Statistical analysis

Linear mixed model analysis was used to examine the association of protein intake at breakfast (g) and protein intake at lunch (g) with total daily protein intake (g, g kg BW^{-1}, g kg adjusted BW^{-1}, g kg FFM^{-1}) at 2 or 3 days, with a random intercept for subject and a random slope for protein intake at breakfast. The random intercept takes into account that subjects provide dietary intake data from multiple days. The random slope is a variance parameter that is estimated from the different slopes, which is included in the model. These models are adjusted for sex, age, BMI, smoking status, study and total energy intake (kcal day^{-1}). Additionally, the association of protein intake at breakfast and protein intake at lunch (g) with protein intake during the rest of the day [total daily protein intake minus protein intake at breakfast or lunch (g)] and protein intake during subsequent meals was studied. Finally, the association of intake of protein source (animal or plant) at breakfast and lunch with total daily protein intake was studied using the same mixed model analysis, with models for animal protein additionally adjusted for plant protein and vice versa.

Effect modification by sex, age, BMI and study was tested for the association between protein intake at breakfast (g) or protein intake at lunch (g) and total daily protein intake (g, g kg BW^{-1}, g kg adjusted BW^{-1}, g kg FFM^{-1}). For most associations sex was an effect modifier; therefore, all analyses were stratified for sex. All analyses were performed using IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA).

Results

Subjects

In total, 498 participants were included into this analysis. Figure 1 shows the number of participants originally included in each study [20-23] and the number of food records days used for this analysis. In total 1477 food record days were included in the analysis. The mean (SD) age of the study population was 67.7 (7.3) years, 42% were male; mean BMI was 30.0 (5.6) kg m^{-2} and 21% were normal weight (BMI 20–25 kg m^{-2}), 30% were overweight (BMI 25–30 kg m^{-2}) and 49% were obese (BMI $\geq$ 30 kg m^{-2}). The general characteristics of the study population are presented in Table 1.

Dietary intake

Mean (SD) energy intake for the total study population was 1898 (526) kcal, with a protein intake of 82 (24) g or 0.97 (0.30) g kg BW^{-1}. Absolute intake of energy and protein was higher for males than for females, whereas protein intake in g kg BW^{-1} day^{-1} and in g kg FFM^{-1} day^{-1} was higher in females (Table 2). In total 70% of the study population reached a protein intake of 0.8 g kg BW^{-1} and 19% reached a protein intake of 1.2 g kg BW^{-1}. Only 1% (n = 4) reached the suggested amount of 0.4 g kg BW

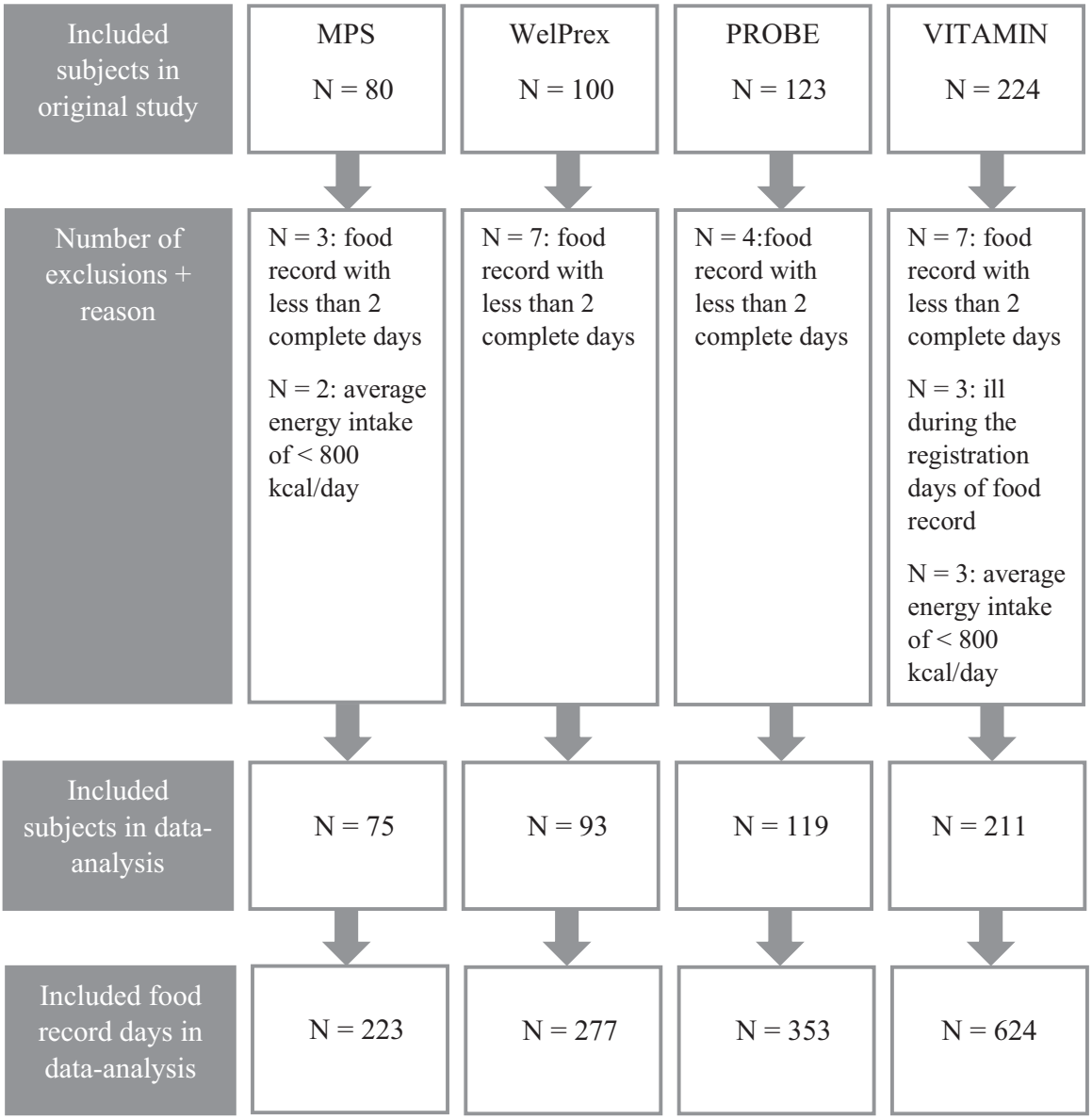

Figure 1 Flow chart for inclusion of baseline data of older adults (n = 498) participating in lifestyle interventions at the Amsterdam Nutritional Assessment Center in the data analysis.

protein^{-1} [28] at breakfast, with 8% and 51% reaching this value at lunch and dinner, respectively. These percentages are higher using adjusted body weight for subjects with a BMI ≥30 kg m^{-2} or <22 kg m^{-2} and all percentages were higher for females compared to males (Table 2). Figure 2 shows the protein and other macronutrient intakes at all eating moments during the day for the total study population. For males and females, the distribution of protein intake over the day was comparable. For males, mean (SD) protein intake was 15.2 (8.2) g at breakfast, 19.9 (10.3) g at lunch and 38.3 (15.5) g at diner. For females, the intakes were 13.0 (6.2), 18.2 (8.7) and 33.9 (13.0) g, respectively.

The within-subject coefficient of variation was 23% for total daily protein intake (g), 32% for protein intake at breakfast (g) and 46% for protein intake at lunch (g).

Association of protein intake at breakfast and lunch with total daily protein intake

Table 3 shows the association of protein intake at breakfast and lunch with total daily protein intake, as well as with protein intake during the rest of the day, adjusted for sex, age, BMI, smoking status, study and total energy intake.

Table 1 Baseline characteristics of older adults participating in lifestyle interventions at the Amsterdam Nutritional Assessment Center

	Total study population (n = 498)		MPS* (n = 75)	WelPrex* (n = 93)	PROBE* (n = 119)	VITAMIN* (n = 211)	P-value[†]
	Mean ± SD/%	Range[‡]	Mean ± SD/%	Mean ± SD/%	Mean ± SD/%	Mean ± SD/%	
Age (years)	67.7 ± 7.3	55–91	63 ± 6	63 ± 5	67 ± 6	72 ± 6	<0.001
% females	58.2%		60.0%	62.4%	33.6%	69.7%	<0.001
Body weight (kg)	86.9 ± 18.5	46.0–146.3	95.4 ± 13.9	92.3 ± 14.5	100.6 ± 15.7	73.7 ± 13.9	<0.001
Height (m)	1.70 ± 0.09	1.50–1.94	1.69 ± 0.09	1.69 ± 0.09	1.73 ± 0.09	1.68 ± 0.09	<0.001
BMI (kg m^{-2})	30.0 ± 5.6	17.5–54.6	33.2 ± 4.4	32.1 ± 4.3	33.6 ± 4.4	25.9 ± 4.2	<0.001
% Overweight[§]	30.3%		24.0%	33.3%	18.5%	37.9%	0.001
% Obese[§]	48.8%		76.0%	65.6%	81.5%	13.3%	<0.001
Waist circumference (cm)	103 ± 15[¶]	66–146	111 ± 11	108 ± 12[††]	115 ± 10[‡‡]	90 ± 11	<0.001
Fat free mass (kg)	51.5 ± 11.9[¶]	28.2–85.3	54.0 ± 10.8**	52.4 ± 12.1[††]	58.5 ± 11.0	46.0 ± 10.0[§§]	<0.001
Fat mass (kg)	35.2 ± 12.2[¶]	9.5–91.3	41.1 ± 10.9**	39.8 ± 9.8[††]	40.6 ± 11.6	27.7 ± 10.0[§§]	<0.001
Body fat percentage (%)	40.0 ± 9.1[¶]	12.6–66.1	43.1 ± 8.6**	43.3 ± 8.4[††]	40.2 ± 8.2	37.2 ± 9.3[§§]	<0.001
% Smoking	7.3%[¶]		9.5%**	8.6%	10.1%	4.3%[§§]	0.180

*The four lifestyle interventions with trial register numbers are the MPS (Muscle Preservation Study): NL2623; the WelPrex (Weight Loss with Protein and Exercise) study: NL4434; the PROBE (protein and lifestyle intervention to preserve muscle mass in obese older type 2 diabetes patients) study: NL4357 and the VITAMIN (VITal AMsterdam older adults IN the city): NL5472 (http://www.trialregister.nl).

[†]*P*-value for differences between the four lifestyle interventions. For nominal variables, Pearson's chi-squared test is used; for continuous variables, one-way analysis of variance is used.

[‡]Range is presented as a minimum to maximum value.

[§]Overweight = body mass index (BMI) ≥ 25 and < 30 kg m^{-2}, obese = BMI ≥ 30 kg m^{-2}.

[¶]*n* waist circumference and *n* smoking status = 495, *n* fat free mass, fat mass and body fat percentage = 479.

**MPS: *n* fat free mass, fat mass and body fat percentage = 70, *n* smoking status = 74.

[††]WelPrex study: *n* fat free mass, fat mass and body fat percentage and waist circumference = 92.

[‡‡]PROBE study: *n* waist circumference = 117.

[§§]VITAMIN study: *n* fat free mass, fat mass and body fat percentage = 198, *n* smoking status = 209.

After adjustment for these potential confounders, a 10 g higher protein intake at breakfast was associated with a 3.2 g higher total daily protein intake ($P = 0.007$) corresponding to a higher total daily protein intake for males of 0.02 g kg BW^{-1} ($P = 0.048$) or 0.03 g kg adjusted BW^{-1} ($P = 0.045$). These associations were stronger for females: a 10 g higher protein intake at breakfast was associated with a 4.9 g higher total daily protein intake ($P < 0.001$) corresponding to a higher total daily protein intake of 0.06 g kg BW^{-1} ($P < 0.001$) or 0.07 g kg adjusted BW^{-1} ($P < 0.001$) (Table 3). However, after adjustment for potential confounders, protein intake at breakfast was significantly negatively associated with protein intake during the rest of the day (total daily protein intake minus protein intake at breakfast): a 10 g higher protein at breakfast was associated with a 6.8 g and 5.1 g lower protein intake during the rest of the day for males and females, respectively. Thus, a 10 g higher protein intake at breakfast did not translate into a 10 g higher total daily protein intake, instead translating into a 3.2 g (males) and 4.9 g (females) higher total intake and therefore a 6.8 g (males) and 5.2 g (females) lower protein intake during the rest of the day (Table 3). A higher protein intake at breakfast was negatively associated with the protein intake at lunch only for males (Table 3). For protein intake at lunch, these associations are in line with the associations for breakfast (Table 3).

When analysing the association of intake of protein source (animal or plant) at breakfast and lunch with total daily protein intake, it appears that this association for plant and animal protein is different. A 10-g higher animal protein intake at breakfast is associated with a 5.6 g (95% confidence interval = 2.7–8.5 g, $P < 0.001$) higher total daily protein intake for males and a 7.6 g (5.2–1.0 g, $P < 0.001$) higher total daily protein intake for females. A 10 g higher plant protein intake at breakfast, however, is associated with a non-significant 0.9 g (−2.6–4.3 g, $P = 0.631$) lower total daily protein intake for males and a 2.7 g (−1.0 – 6.5 g, $P = 0.156$) lower intake for females, as well as a significant lower protein intake during the rest of the day, including lunch and dinner. Associations for the source of protein intake at lunch with total daily protein intake, and with protein intake during the rest of the day were in line with the associations described for breakfast.

Table 2 Average dietary intake per day* of older adults participating in lifestyle interventions at the Amsterdam Nutritional Assessment Center

	Total study population *n* = 498		Males *n* = 208	Females *n* = 290
	Mean ± SD, or %	Range†	Mean ± SD	Mean ± SD
Energy (kcal)	1898 ± 526	800–4069	2021 ± 521	1810 ± 512
Energy (kJ)	7958 ± 2200	3356–17073	8473 ± 2181	7589 ± 2142
Total protein intake (g day^{-1})	82 ± 24	25–215	88 ± 27	77 ± 23
Plant protein intake (g day^{-1})	29 ± 10	8–72	31 ± 11	28 ± 9
Animal protein intake (g day^{-1})	52 ± 20	5–155	56 ± 20	50 ± 20
Fat intake (g day^{-1})	74 ± 28	15–196	78 ± 27	71 ± 29
Carbohydrate intake (g day^{-1})	195 ± 62	51–443	206 ± 64	186 ± 59
Protein intake energy%	17.6 ± 3.6	8.6–33.4	17.7 ± 3.4	17.5 ± 3.8
Fat intake energy%	34.6 ± 6.8	13.3–59.0	34.4 ± 6.5	34.8 ± 7.0
Carbohydrate intake energy%	41.2 ± 7.3	19.0–75.6	40.9 ± 6.9	41.5 ± 7.6
Protein intake (g kg BW^{-1} day^{-1})	0.97 ± 0.30	0.30–2.33	0.93 ± 0.27	0.99 ± 0.31
Protein intake (g kg adj‡ BW^{-1} day^{-1})	1.07 ± 0.31	0.37–2.40	1.04 ± 0.28	1.09 ± 0.32
Protein intake (g kg FFM^{-1}§ day^{-1})	1.64 ± 0.52	0.55–4.29	1.41 ± 0.37	1.81 ± 0.54
% with intake ≥ 0.8 g kg BW^{-1} day^{-1}	70%		67%	73%
% with intake ≥ 1.2 g kg BW^{-1} day^{-1}	19%		15%	21%
% with intake ≥ 0.8 g/kg adj‡ BW^{-1} day^{-1}	83%		81%	85%
% with intake ≥ 1.2 g kg adj‡ BW^{-1} day^{-1}	29%		27%	31%
% consuming ≥ 0.4 g kg BW^{-1} at breakfast	1%		0%	1%
% consuming ≥ 0.4 g kg BW^{-1} at lunch	8%		7%	10%
% consuming ≥ 0.4 g kg BW^{-1} at dinner	51%		46%	56%
% consuming ≥ 0.4 g kg adj‡ BW^{-1} at breakfast	2%		1%	2%
% consuming ≥ 0.4 g kg adj‡ BW^{-1} at lunch	10%		9%	10%
% consuming ≥ 0.4 g kg adj‡ BW^{-1} at dinner	63%		60%	65%

*Average dietary intake is calculated from the mean intake per day of each subject (*n*= 498).

†Range is presented as a minimum to maximum value.

‡Using adjusted body weight for obese subjects [using body weight at body mass index (BMI) 27.5 kg m^{-1}] [23] and for subjects with a BMI < 22 kg m^{-2} (using body weight at BMI 22 kg m^{-2}) [24].

§Fat free mass (FFM) assessed using air displacement plethysmography (BODPOD, Life Measurement Inc.), *n* total study population = 479, *n* female = 277, *n* male = 202.

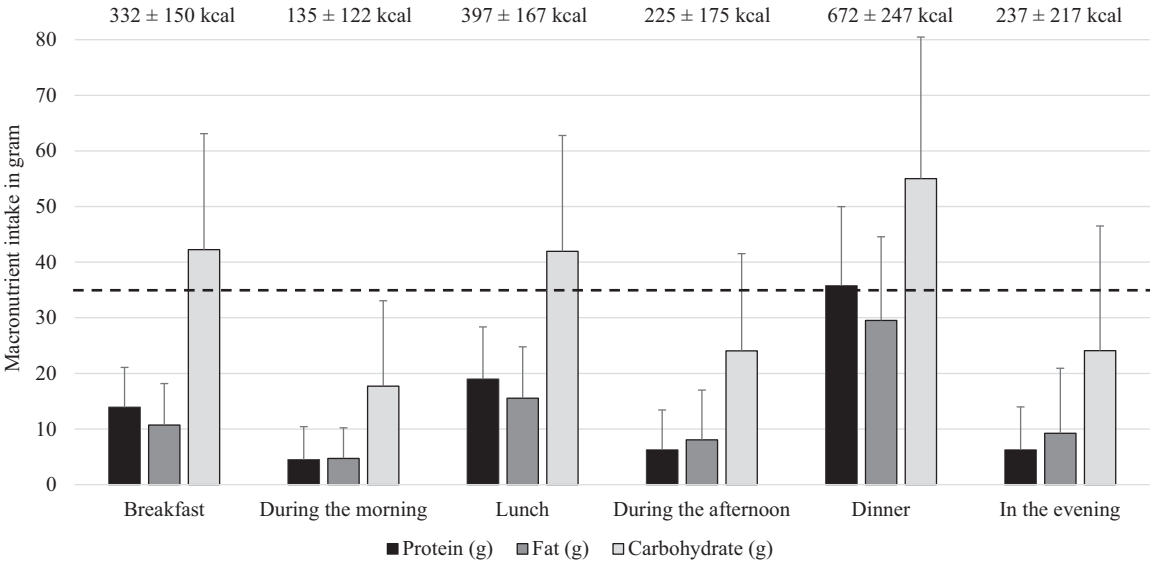

Figure 2 Macronutrient intake per meal. The bars represent an average macronutrient intake per eating moment over the 3-day food records (*n* = 498). The dashed line represents the amount of protein per meal that is suggested to stimulate protein synthesis[28], as calculated using the average body weight of the study population.

Table 3 Associations* of protein intake at breakfast and lunch (g day^{-1}) with total daily protein intake, and with protein intake during the rest of the day† and subsequent meals‡ in older adults

	Males (n = 208)			Females (n = 290)		
	Beta	95% CI	P-value	Beta	95% CI	P-value
Associations of protein intake at breakfast in g day^{-1}(independent variable)						
Total protein intake (g day^{-1}) (dependent variable)						
Crude model§	0.90	0.59–1.20	<0.001	1.09	0.82–1.36	<0.001
Adjusted model§	0.32	0.09–0.56	0.007	0.49	0.27–0.70	<0.001
Total protein intake (g kg body weight^{-1} day^{-1}) (dependent variable)						
Crude model§	0.007	0.004–0.010	<0.001	0.010	0.007–0.013	<0.001
Adjusted model§	0.002	0.000–0.005	0.048	0.006	0.003–0.009	<0.001
Total protein intake (g kg adjusted body weight^{-1} day^{-1}) (dependent variable)						
Crude model§	0.009	0.005–0.012	<0.001	0.015	0.011–0.018	<0.001
Adjusted model§	0.003	0.000–0.006	0.045	0.007	0.004–0.010	<0.001
Total protein intake (g kg FFM^{-1}¶ day^{-1}) (dependent variable)						
Crude model§	0.012	0.007–0.016	<0.001	0.021	0.014–0.028	<0.001
Adjusted model§	0.004	−0.000 – 0.007	0.068	0.011	0.005–0.016	<0.001
Protein intake during the rest of the day (g day^{-1})† (dependent variable)						
Crude model§	−0.10	−0.41–0.20	0.497	0.09	−0.18 – 0.36	0.496
Adjusted model§	−0.68	−0.91 – −0.45	<0.001	−0.51	−0.73 – −0.30	<0.001
Protein intake at lunch (g day^{-1}) (dependent variable)						
Crude model§	−0.08	−0.19 – 0.04	0.191	−0.00	−0.13 – 0.13	0.952
Adjusted model§	−0.19	−0.30 – −0.08	0.001	−0.06	−0.19 – 0.08	0.412
Protein intake at dinner (g day^{-1}) (dependent variable)						
Crude model§	0.12	−0.15 – 0.39	0.397	0.21	0.00 – 0.41	0.048
Adjusted model§	−0.08	−0.28 – 0.13	0.462	−0.12	−0.30 – 0.07	0.228
Associations of protein intake at lunch in g day^{-1}(independent variable)						
Total protein intake (g day^{-1}) (dependent variable)						
Crude model§	0.78	0.60–0.96	<0.001	0.98	0.81–1.15	<0.001
Adjusted model§	0.37	0.24–0.51	<0.001	0.58	0.46–0.70	<0.001
Total protein intake (g kg body weight^{-1} day^{-1}) (dependent variable)						
Crude model§	0.007	0.005–0.009	<0.001	0.012	0.010–0.015	<0.001
Adjusted model§	0.003	0.002–0.005	<0.001	0.008	0.006–0.009	<0.001
Total protein intake (g kg adjusted body weight^{-1} day^{-1}) (dependent variable)						
Crude model§	0.009	0.006–0.011	<0.001	0.013	0.011–0.016	<0.001
Adjusted model§	0.004	0.002–0.006	<0.001	0.008	0.006–0.009	<0.001
Total protein intake (g kg FFM^{-1}¶ day^{-1}) (dependent variable)						
Crude model§	0.011	0.008–0.014	<0.001	0.023	0.019–0.028	<0.001
Adjusted model§	0.005	0.003–0.007	<0.001	0.014	0.011–0.016	<0.001
Protein intake during the rest of the day (g day^{-1})† (dependent variable)						
Crude model§	−0.22	−0.40 – −0.04	0.020	−0.02	−0.19 – 0.15	0.817
Adjusted model§	−0.63	−0.76 – −0.49	<0.001	−0.42	−0.54 – −0.30	<0.001
Protein intake at dinner (g day^{-1}) (dependent variable)						
Crude model§	−0.00	−0.13 – 0.13	0.968	0.14	−0.00 – 0.28	0.054
Adjusted model§	−0.19	−0.32 – −0.06	0.005	−0.10	−0.20 – 0.01	0.074

CI, confidence interval.

*For associations with independent variable protein intake at breakfast: analysed with linear mixed models with a random intercept for subject and a random slope for protein intake at breakfast, n = 1477 food record days; for associations with independent variable protein intake at lunch: analysed with linear mixed models with a random intercept for subject and a random slope for protein intake at lunch, n = 1477 food record days.

†For associations with independent variable protein intake at breakfast: protein during the rest of the day (g) = daily protein intake (g) – protein intake at breakfast (g); for associations with independent variable protein intake at lunch: protein during the rest of the day (g) = daily protein intake (g) – protein intake at lunch (g).

‡For associations with independent variable protein intake at breakfast: subsequent meals are lunch and dinner; for associations with independent variable protein intake at lunch: subsequent meal is dinner.

§The crude model is the model without adjustments; the adjusted model adjusted for sex, age, body mass index, smoking status (current smoker, yes/no), study and total energy intake.

¶Fat free mass (FFM) is assessed using air displacement plethysmography (BODPOD, Life Measurement Inc.), n = 1420 food record days.

Discussion

The present study investigated the association between protein intake at breakfast and lunch with the total daily protein intake among older adults and demonstrates that a higher protein intake at breakfast and lunch is associated with a lower protein intake during the rest of the day (total daily protein intake minus breakfast) but, overall, with a higher total daily protein intake.

In our study population, less than 30% met the suggested recommendation of 1.2 g protein kg BW^{-1} [29,30] using adjusted body weight [26,27]. Having a higher protein intake at breakfast (≥30 g) was associated with more subjects reaching 1.2 g protein kg BW^{-1}: 52% versus 28% of the subjects. For lunch, these percentages were 61% versus 25% of the subjects. These findings are in line with the study of Tieland *et al.* [12], in which an even protein distribution over the day, with more protein at breakfast and lunch, was associated a higher percentage of subjects achieving the recommended daily allowance of 0.8 g kg BW^{-1} day^{-1}.

Because the present study has a cross-sectional design, no suggestions for a causal relationship can be made. The study, however, does give an indication that a higher protein intake at breakfast and lunch might have a satiating effect because protein intake at both breakfast and lunch was negatively associated with protein intake during the rest of the day. The total daily protein intake, however, was not compromised and a higher protein intake at breakfast and lunch was still related to a higher total protein intake. However, a higher plant protein intake at breakfast and lunch was not associated with a higher total daily protein intake, in contrast to animal protein. This might suggest that plant protein sources have a stronger satiating effect, although this proposal should be considered with caution because other factors such as the food form also play a role. For example, animal protein might be consumed in more liquid forms (e.g. milk or yoghurt), which probably suppresses appetite less compared to solid forms [31], although this requires further study. Lonnie *et al.* [31] reported that a higher consumption of plant proteins found in whole food also increases dietary fibre, which might amplify satiety. Data regarding the effects of plant proteins on appetite in older adults, however, are very limited and should be investigated in future studies, in addition to the food groups, food form and the food matrix [31].

To our knowledge, the present study is the first to investigate the association between regular protein intake at breakfast and lunch and total daily protein intake. Hengeveld *et al.* [18] demonstrated that older adults (>70 years) with an adequate protein intake (≥0.8 g kg^{-1}) had higher protein intakes at all eating occasions, including breakfast and lunch, which is in line with our findings. Several other studies demonstrate that the use of protein enriched meals or foods does not limit and mostly increases the amount of protein per meal and total daily protein intake in older adults [32-34]. This indicates that satiating effects of higher protein meals or foods are limited in older adults [34]. Giezenaar *et al.* [35] showed that although gastric emptying was slower in older compared to younger men, which gives a prolonged post-prandial satiety, the acute administration of whey protein drinks before a meal suppressed subsequent energy intake in young, but not in healthy older men. These findings were substantiated by Clegg *et al.*[36].

Only 2% and 10% of our subjects reached the suggested amount of 0.4 g kg $protein^{-1}$ [28] at breakfast and lunch, which suggests that habitual protein intake during breakfast and lunch is generally low. The range of habitual protein intake at breakfast and lunch in the present study, however, is large and is achieved with regular food products. This shows that a higher protein intake at breakfast is achievable for some older adults and also demonstrates potential for improvement. A higher protein intake at breakfast and lunch may lead to a higher number of eating occasions that reach the suggested anabolic threshold for optimal muscle protein synthesis [28]. Regardless of the total daily protein intake, this is already a potential gain, which might impact subsequent muscle maintenance or accretion [6] and is important with respect to preventing or counteracting sarcopenia. However, this has not yet been substantiated by long-term dietary intervention trials.

A limitation of the present study is the high percentage of obese older adults (almost 50%) in our study population. Obese adults have a higher prevalence of carrying the specific single nucleotide polymorphisms in the fat mass and obesity-associated gene (FTO) [37]. FTO might facilitate weight gain by decreasing the release of the satiety hormone leptin and increasing the release of hunger-promoting hormone ghrelin [38]. Therefore, the satiating effect of a meal might be lower in obese subjects. The representativeness of the study population compared to the general older population may thus be low. In the present study, however, we did not observe differences in the association of protein intake at breakfast and lunch with total daily protein intake between obese and non-obese subjects. This suggests that potential differences in the release of hunger and satiety hormones for obese versus non-obese subjects do not appear to translate into differences in the relationship between protein intake at breakfast or lunch and protein intake during the rest of the day. A lower protein intake at breakfast, however, was related to a lower BMI: the 10% of the participants with the lowest protein intake at breakfast had a significantly

lower BMI than subjects with a higher protein intake at breakfast [28.3 (4.8) versus 30.2 (5.6) kg m^{-2}]. Because BMI was also related to the primary outcome total daily protein intake, all models were adjusted for BMI. Another limitation concerns the reported energy intake in the present study, which is comparable to that for Dutch older adults in general [18], whereas almost half of our study population was obese. We therefore expected a higher energy intake in our study population. Based on previous research [39], overweight people tend to underestimate their dietary intake more often than normal-weight people, and therefore true energy and protein intake could be underestimated in our study. Park *et al.* [40] demonstrated that a dietary food record has advantages compared to a food frequency questionnaire: less under-reporting of energy and nutrients. In both overweight and obese subjects, protein intake with a dietary record was less under-reported than energy intake. A third limitation is that we did not adjust for the potential confounding factors education-level and income [41] because these variables were not available for all included studies. A final limitation is that our study population had a wide age range, from 55 to over 90 years. Although age was no effect modifier in the relationship between protein intake at breakfast or lunch and total daily protein intake, the dietary intake of food groups and the dietary pattern may change during the ageing process as a result of a wide variety of factors [42].

The present study also has some strengths. We used a 3-day dietary food record to assess protein intake, which probably gives a more realistic estimate of dietary intake than a recall-method in this older population because it is likely to be less prone to short-term memory loss. In addition, we used a linear mixed model analysis that took into account the within-subject, day-by-day variation of dietary intake, which provides a more sensitive analysis than using an average dietary intake per subject.

Conclusions and implications

In conclusion, a higher protein intake at breakfast and lunch is associated with a higher total daily protein intake in community dwelling older adults. This association holds true for animal protein, although not for plant protein for which no association was observed. In sum, stimulating a higher protein intake at breakfast and lunch might represent a promising nutritional strategy for optimising the amount of protein per meal without compromising total daily protein intake.

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Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.
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JvdH, CvD, RGM and AMV conducted the included studies (hands-on conduct of the experiments and data collection). AMV, MTS, IR, DH, MT and PJMW analysed and/or checked the statistical analysis. AMV, JvdH, MTS, IR, DH, MT, MFE, MV and PJMW wrote and/or revised the manuscript. PJMW had primary responsibility for the final content. All authors critically reviewed the manuscript and approved the final version submitted for publication.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with STROBE guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

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CARDIOVASCULAR DISEASE

Potential curing and beneficial effects of Ooitabi (*Ficus pumila* L.) on hypertension and dyslipidaemia in Okinawa

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Keywords
Ooitabi, *Ficus pumila* L, hypertension, dyslipidaemia, flavonoid.

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Abstract

Background: Over 30% of the population of Okinawa Prefecture have a high body mass index. The incidence of hypertension and dyslipidaemia has also increased in recent years. We found that Ooitabi (*Ficus pumila* L.), a plant native to Okinawa, was useful for hypertension. During ancient times, the extracts of Ooitabi leaves were used for making Ishimaki tea in some areas of Okinawa Prefecture. The plants in Okinawa are rich in antioxidants, and four flavonoid glycosides, including rutin, have been identified in Ooitabi.
Methods: In the present study, we conducted clinical verification tests on the effects of drinking Ishimaki tea on outpatients with hypertension and dyslipidaemia. Of 3814 Japanese patients who underwent medical check-ups in Okinawa, 38 individuals with high blood pressure, dyslipidaemia, liver dysfunction and gout visited our hospital as outpatients and were asked to drink Ishimaki tea.
Results: After 3 months, there were significant reductions in body mass index, systolic and diastolic blood pressure, total cholesterol, low-density lipoprotein cholesterol, γ-glutamyltrans peptidase, uric acid and ratio of blood vessel insulin resistance.
Conclusions: Ooitabi extract can lower blood pressure and improve lipid abnormalities and has likely contributed to the well-known health and longevity of the population in Okinawa.

Introduction

Of 3814 individuals who underwent health check-ups at our hospital in Okinawa Prefecture from January 2019 to September 2019, 1432 (37.5%) had a high body mass index (BMI) (≥25 kg m^{-2}), 1408 (36.9%) had high systolic blood pressure (sBP) (≥130 mmHg), 1019 (26.7%) had high diastolic blood pressure (dBP) (≥85 mmHg), 792 (27.4%) had high total cholesterol (TC) (≥220 mg dL^{-1}), 1468 (46.0%) had high low-density lipoprotein cholesterol (LDL-C) (≥120 mg dL^{-1}), 1462 (45.8%) had high γ-glutamyl trans peptidase (γ-GTP) (≥50 IU L^{-1}), 580 (24.6%) had high uricacid (UA) (≥7.1 mg dL^{-1}), 741 (23.2%) had high triglyceride (TG) (≥150 mg dL^{-1}), 207 (6.4%) had low high-density lipoprotein cholesterol (HDL-C) (≤39 mg dL^{-1}) and 695 (21.8%) had high vascular insulin resistance defined as a TG/HDL ratio >3. In Okinawa Prefecture, the percentage of people who have high BMI, high BP and dyslipidaemia has reached 30%. Despite the availability of drugs for treatment, several of these individuals have not started any pharmacotherapy. Therefore, the management of the health of such individuals is a major issue and should entail specific health check-ups in Okinawa Prefecture and health guidance,

according to the implementation plan of the Naha City Health Department Specific Health Checkup Division (i.e. Naha City second phase insurance business operation plan or data health plan). To enhance the effectiveness of the plan, Healthy Naha 21 (second phase) was designed as an insurance and welfare plan comprising a review of eating habits. Notably, the population of Okinawa Prefecture is known to have longevity and traditional eating habits [1]. Okinawa is also known for its people's long lifespans [2,3]. One contributing factor is the ingestion of a group of locally grown plants, called island vegetables, from ancient times onwards. As a result of its subtropical location, Okinawa enjoys a warm climate all year round. Perhaps because of this climate, the plants are extremely diverse, and there are many heteromorphic plants of primary colors, which protect them from ultraviolet radiation. The plants are known to produce phytochemicals [4], which are non-nutritional physiologically active substances contained in plants, such as vegetables, fruits and grains, and include polyphenols, organic sulphur compounds and carotenoids. Although these plant-derived compounds are not required to maintain physical function, they may have a positive influence on health [5].

In Okinawa, it is reported that people have been drinking the extraction of Ooitabi plant for their health for more than 500 years. However, the antihypertensive and fat-lowering benefits of Ooitabi leaves have not been confirmed clinically. Epidemiological studies of the health relevance with respect to anti-metabolism and Ooitabi polyphenol consumption still remain outstanding.

The present study is focused on the effects of Ooitabi leaves on hypertension and dyslipidaemia among Japanese patients who underwent medical check-ups in Okinawa Prefecture.

Materials and methods

Study setting

From ancient times onwards, elderly individuals with high BP living in the northern part of the Okinawa Main Island (Figure 1) have been drinking Ishimaki tea, which was made by drying the stems and leaves of Ooitabi, followed by extraction of the major components with water. Ishimaki tea is said to have antihypertensive effects, and its name is derived from the fact that Ooitabi winds up (maki-tsuku) on stone walls (Ishi-gaki). An Ooitabi plant winding up stone walls is shown in Figure 2a; its leaves are green but turn white after drying for 1 day. When 20–25 g of the dried leaves are placed in 1 L of boiling water at 100 °C for 7 min, a brown liquid is produced (Figure 2b), which is mixed with a flavour and heated for approximately 2 min to yield the more palatable Ishimaki tea compared to the original extract.

Participants and study design

Among the 3814 Japanese patients who underwent medical check-ups in Okinawa Prefecture, volunteers (n = 38) with upper borderline high BP and dyslipidaemia were asked to drink approximately 200–300 mL of Ishimaki tea a day.

Figure 1 Vegetation and location of Ooitabi. Ooitabi grows in Nago City of Northern Okinawa. This is from the official homepage of the Japanese Municipality of Okinawa Prefecture (Download Brochure/Official Website of Okinawa Prefecture; outline of Okinawa Prefecture; https://www.pref.okinawa.jp/site/chijiko/kohokoryu/foreign/english/download.html.

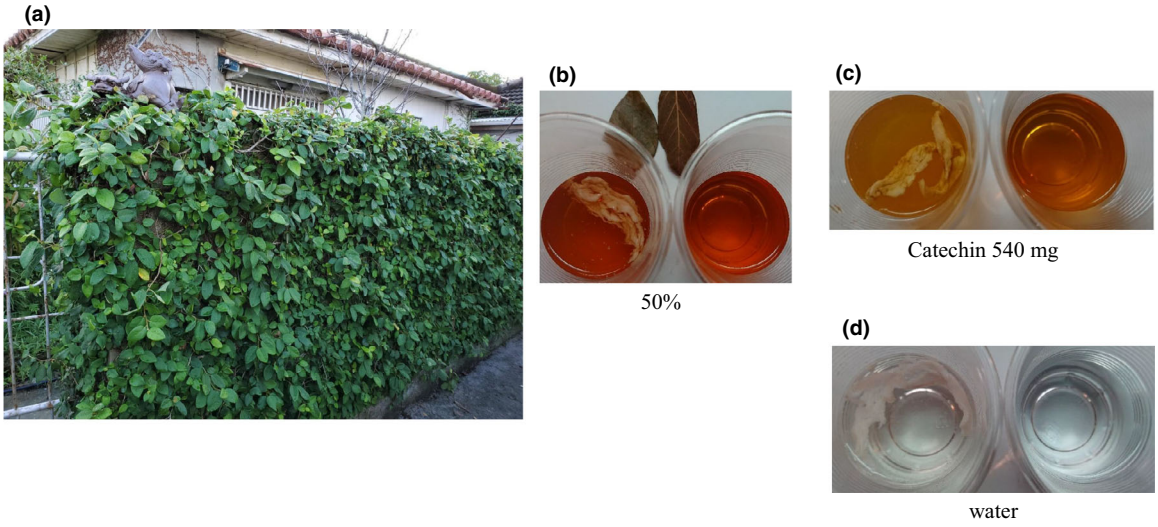

Figure 2 Vegetation and extraction of Ooitabi and subcutaneous fat from an Au pig. Ooitabi grows on stone walls (a). After collection, the dried Ooitabi leaves are then extracted with steeped in hot water. For 24 h, the subcutaneous fat was left in commercially available cups containing Ooitabi extracts at 50% (b), drinking water containing catechin at concentrations of 540 mg per 500 mL (c) and pure water (d). The cups on the left contain fat, whereas those on the right contain the reference solution without fat. (b–d). An Ooitabi leaf after extraction. The cups with Ooitabi extract (b) and catechin (c) have almost similar levels of dissolved fat.

These volunteers did not seek treatment on their own but were outpatients who were undergoing periodic medical examinations, comprising blood collection and follow-up. Moreover, these outpatients had never consumed Ishimaki tea or had taken consumed it in the remote past. After 3 months of drinking Ishimaki tea, the participants were re-evaluated with respect to BMI and hypertension, and underwent blood sampling for measurement of TC, LDL-C, γ-GTP, UA, TG and HDL-C levels. All participants provide their written informed consent forms to disclose the results and study details in scientific articles.

Source of plants and plant specimens

Ficus pumila was introduced to western science in 1721. It was first described and named as *Ficus stipulata* in 1753 by Carl Linnaeus. The species name 'pumila' is a derivation of the Latin word for 'dwarf'. This refers to the juvenile growth, which bears small, ovate or heart-shaped leaves of no more than 2.5 cm [6]. There are more than 850 members of the *Ficus* genus; among them are several species commonly available for indoor gardeners, including the creeping fig, or *F. pumila* (sometimes also called *Ficus repens*) [7]. The *Ficus* genus contains some of the most beautiful, widespread and important plants in the world. The typification of the name *Ficus pumila* var. *pumila* (*Moraceae*) is discussed. The designation of the corresponding type is based on the consultation of Linnaeus's original material and the literature cited in the protologue [8]. Similar to other plant species in the family Moraceae, contact with the milky sap of *Ficus pumila* can cause phytophotodermatitis, a potentially serious skin inflammation [9].

However, stems and leaves of *Ficus pumila* L. have been used in Traditional Chinese Medicine as a tonic and to treat fever. Leaves of *Ficus pumila* L. are used in Japan in beverages to treat diabetes and high blood pressure [10]. *Ficus pumila* L. is native to East Asia, and also inhabits warm areas, such as Okinawa. *Ficus pumila* L. has been known as 'Ooitabi' in Okinawa from ancient time onwards.

These plants have been reported to have antioxidant activity [11]. As shown in Figure 2a, the plant (Ooitabi) grows on stone walls of any civilian houses of Nago area (i.e. Nago City) (Figure 1). Thus, seeds were not obtained commercially, nor were they acquired from another laboratory. Because these samples(plants) were obtained from the stone walls of civilian houses (from the wild), special permission was not necessary to collect the samples (other than thanking the house owners). Because plants (Ooitabi) on stone walls have been collected for a long time by civilians for making tea, there are institutional or national guidelines regarding the usage of such plants. Therefore, special permissions and/or licenses are not necessary.

Description of materials

The ripe fruit pod taken from the female strain of Ooitabi has a sweet and mellow taste and has been said to be sweeter than figs. Ooitabi fruits have been shown to suppress nitric oxide production in diabetes and cancer and to exert an antioxidant action in the 2,2-diphenyl-1-picryl

hydrazyl radical scavenging effect test [12]. However, eating the fruit does not appear to be a common practice. On the other hand, Ooitabi stems and leaves have been widely consumed for tonicity, common cold and diabetes. Four types of flavonoids, including apigenin 6-neohesperidosy, kaempferol 3-robinobioside, kaempferol 3-rutinoside [13,14,15,16,17] and rutin, can be extracted from Ooitabi leaves and had been shown to have antioxidant actions and may further improve diabetes [18].

The study was reviewed and approved by the Daido Central Hospital. IRB and informed consent were obtained from each subject prior to their participation in the study. Statistical analysis was only conducted if the variation with a treatment (SD divided by the means) was greater than 10% and the difference among treatment means was less than 3 SDs.

Results

The clinical effect of Ishimaki tea

As shown in Figure 3, drinking Ishimaki tea for 3 month significantly reduced mean (SD) body mass index [29.0 (5.8) versus 22.9 (3.1) mmHg, $P < 0.001$]; systolic BP [142.0 (8.6) versus 126.3 (10.6) mmHg, $P < 0.001$]; diastolic BP [91.4 (9.7) versus 85.7 (8.1) mmHg, $P = 0.007$]; TC [235.0 (24.9) versus 210.6 (22.4) mg dL^{-1}, $P < 0.001$]; LDL-C [147.6 (28.6) versus 135.4 (24.5) mg dL^{-1}, $P = 0.048$]; γ-GTP [117.5 (73.9) versus 89.0 (47.7) mg dL^{-1}, $P = 0.05$]; UA [8.3 (1.2) versus 6.6 (1.1) mg dL^{-1}, $P < 0.001$]; TG [239.4 (56.8) versus 171.3 (36.6) mg dL^{-1}, $P < 0.001$]; and TG/HDL-C [5.5 (2.0) versus 3.3 (0.9) mg dL^{-1}, $P < 0.001$], whereas HDL was significantly increased [46.2 (11.2) versus 52.3 (8.7) mg dL^{-1}, $P = 0.011$].

The biological effect of Ishimaki tea

In another experiment (Figure 2), the subcutaneous fat of an Agu pig from Okinawa Prefecture was immersed in either Ooitabi extracts, catechin or pure water, followed by observation of the degree of turbidity over time. In the liquid containing the Ooitabi extract, a significant portion of the fat dissolved and the liquid became turbid after 24 h. The liquid with 50% Ooitabi extract yielded almost the same turbidity as that of the liquid containing 540 mg per 500 mL of catechin. The extracted (100%) Ooitabi liquid was very opaque and cloudy, precluding any comparison of its gross and photographic appearance with those of the other stock solutions.

Discussion

Among these health check-up patients with hypertension, 44% appeared to be have hereditary hypertension. In particular, some cases with a family history of hypertension had already acquired high BP by the age of 40 years. One 23-year-old man who had hypertension had a family history of the condition in his grandparents and parents. Although the number of cases was small, Ooitabi extracts have the potential to prevent hypertension, even the familial type. Future verification is necessary to confirm this.

Although not very significant, intake of Ooitabi extracts effected some improvements in dyslipidaemia. The 38 participants with dyslipidaemia in the present study found it difficult to take medication, practice good nutritional intake and exercise. Fortunately, drinking Ishimaki tea clearly lowered their cholesterol levels. Although the mechanism is unknown, the antioxidant action of the Ooitabi extracts might have contributed. Notably, in these cases, the reductions in LDL-C and γ-GTP were minimal. However, the significant increase in HDL-C likely reduced insulin vascular resistance [19]. These findings implied that drinking Ishimaki tea may prevent arteriosclerosis and decrease the risk for diabetes. It was also considered to be useful for the increasing number of dialysis patients in Okinawa.

To convince people to drink Ishimaki tea in their daily life, the degree of fat dissolution was evaluated in a simple cup and was made easy to understand. After soaking for 24 h, the Agu pig fat dissolved in the liquid containing the Ooitabi extracts, making the liquid appear turbid. The degree of turbidity was the same between a 50% concentration of Ooitabi extracts and a catechin-containing tea, which is sold in the market at a concentration of 540 mg per 500 mL. When immersed in pure water, the fat did not dissolve and the water did not become turbid.

Although the degree of turbidity was not quantified, Okinawa people usually drink Ishimaki tea with a 50% Ooitabi extract concentration. *Ficus pumila* L. contains flavonoids such as apigenin, kaempferol and rutin. There are some reports that flavonoids regulate lipolysis. Flavonoids constitute a major group of polyphenolic compounds that are directly associated with the organoleptic and health-promoting properties of red wine. The way that wine flavonoids may be absorbed and metabolised could interfere with their bioavailability and therefore in their health-promoting effect [20]. Black tea polyphenols exert a positive effect with respect to inhibiting obesity via major mechanisms: promoting lipid metabolism by activating AMP-activated protein kinase to attenuate lipogenesis and enhance lipolysis, and decreasing lipid accumulation by inhibiting the differentiation and proliferation of preadipocytes [21]. The mechanisms involved in weight loss in which polyphenols may have a role are: activating β-oxidation; a prebiotic effect for gut microbiota; inducing satiety; stimulating energy expenditure by inducing thermogenesis in brown adipose tissue; modulating adipose tissue inhibiting adipocyte

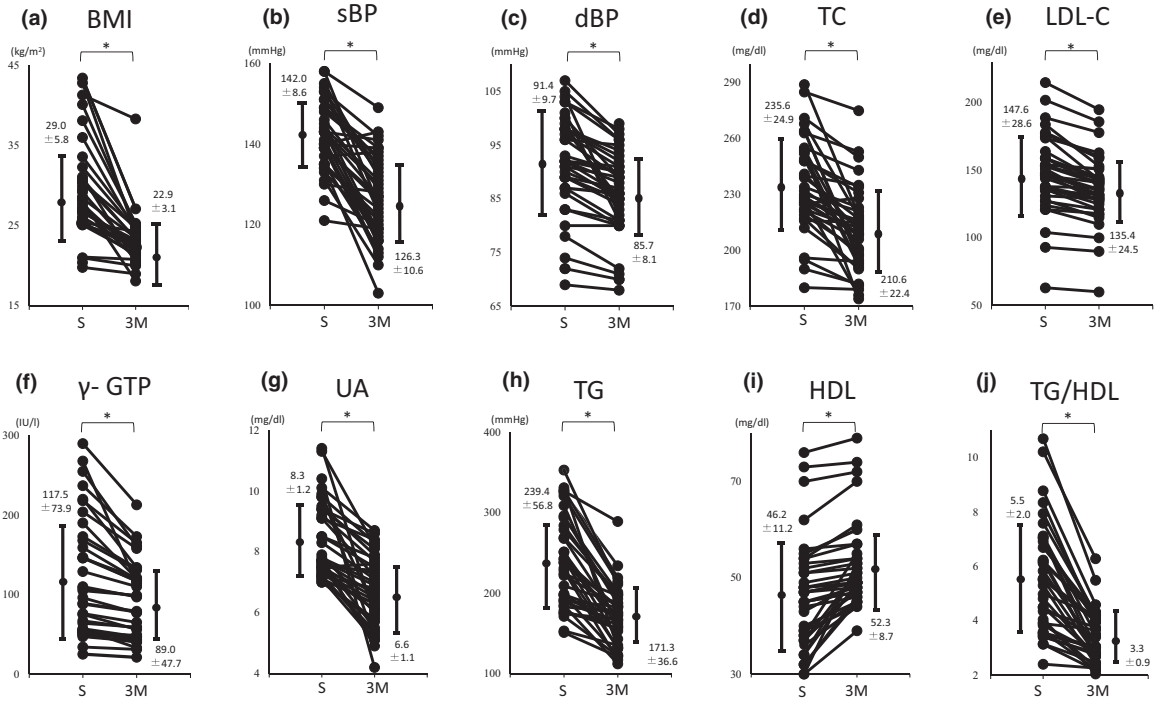

Figure 3 Results after drinking Ooitabi extracts. BMI (a), sBP (b), dBP (c), TC (d), LDL-C (e), γ- GTP (f), UA (g), TG (h) and TG/HDL (j) significantly decreased. HDL-C (i) increased. S, start date; 3M, After 3 months; sBP, systolic blood pressure; dBP, diastolic blood pressure; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; γ-GTP, γ-glutamyl trans peptidase; UA, uric acid; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; TG/HDL, triglyceride to high-density lipoprotein cholesterol ratio. Data are presented as the mean (SD). *P* values were determined using Student's *t* test.

differentiation; and promoting adipocyte apoptosis and increasing lipolysis [22]. It was reported that almond skin polyphenol extract significantly promoted phosphorylation of AMP-activated protein kinase, increased the activity of adipose triglyceride lipase and hormone-sensitive lipase, inhibited adipogenesis-related transcription factors, and regulated lipolysis [23].

In conclusion, a possible reason for the longevity exhibited by those living in Okinawa Prefecture has been reported [24]. Ooitabi is a wild plant that is native to Okinawa, although it is difficult to cultivate, being time consuming and labor intensive, with low-volume productivity and an unpalatable taste. Therefore, not all Okinawa people drink Ishimaki tea. Nevertheless, Ooitabi has possibly contributed to longevity from ancient times onwards. Therefore, the tradition or old practice of drinking Ishimaki tea should be preserved and carried on, aiming to protect health and achieve a longevity of over 100 years old not only for the population of Okinawa and Japan, but also for the general population worldwide, where the incidence of high blood pressure and dyslipidaemia, as well as diabetes and hyperuricemia, has been increasing.

Study strengths and limitations

There is still a great difference between Ooitabi flavonoid bioavailability and their health-promoting effects. More *in vivo* studies focused on flavonoid metabolites are still required. From this verification alone, whereas drinking Ishimaki tea apppears to reduce the amounts of subcutaneous and visceral fat tissues. The present study had some limitations. The absorption rates of the ingested components of the Ishimaki tea (i.e. Ooitabi extracts) were not measured. Therefore, the mechanisms of action off lavonoids and rutin on blood pressure and dyslipidaemia need to be clarified in future studies. Moreover, the quality and quantity of each Ooitabi extract component was not clear. Nevertheless, the appropriate extraction time and concentration were followed, according to the methods used from ancient times onwards in Okinawa. Although the number of participants was small, the present study clearly showed that the Ooitabi extracts lowered the blood pressure of health check-up patients who had borderline hypertension and dyslipidaemia but in whom medical treatments are not yet indicated according to the hypertension guidelines.

Conclusions

Drinking Ishimaki tea clearly lowered BMI, sBP, dBP, TC, LDL-C, γ-GTP, UA, TG, HDL-C and TG/HDL serum levels and increased HDL-C serum levels. The antioxidant action of the Ooitabi extracts might play an important role in the development of blood pressure, fat metabolism and gout. The effect of Ooitabi on metabolic syndrome strongly depends on the extents of high BP, hyperlipidaemia and hyperuricemia that occur with the life style disease.

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Conflicts of interest, source of funding and authorship

The authors declare that they have no conflicts of interest.
No funding declared.
All authors contributed to the research protocol. KS, KG, Y Kishimoto, Y Katsumoto and ST designed the study. KS, KG and Y Kishimoto collected the Ooitabi stems and leave bank data. KS, KG and Y Katsumoto analysed the data and drafted the manuscript. All authors have read and approved the final version of the manuscript submitted for publication.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with STROBE guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

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CARDIOVASCULAR DISEASE

Anthropometric and blood pressure changes in patients with or without nutritional counselling during cardiac rehabilitation: a retrospective study

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Keywords
blood pressure, cardiac rehabilitation, cardiovascular risk, dietitian, weight management.

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Abstract

Background: Whether a patient's outcomes are better when receiving nutritional counselling during cardiac rehabilitation (CR) has been scarcely described. We compared changes in weight, waist circumference (WC) and blood pressure (BP) in patients attending CR with and without nutritional counselling.

Methods: A retrospective analytical study was conducted in which two groups of patients who completed a phase II CR (36 sessions) were compared: CONTROL [n = 144, mean (SD) age = 59 (12) years, 17% females], comprising patients without nutritional counselling (attended between 2003 and 2009), and NUT [n = 128, mean (SD) age = 60 (13) years, 27% females], comprising patients with dietitian-delivered nutritional counselling (attended between 2010 and 2019). Repeated-measures analysis of variance was used to compare changes in weight, WC, and BP during CR between groups. Logistic regression models determined the probability of reducing weight and systolic BP (SBP).

Results: NUT group decreased weight [−1.3 (3.1) kg; $P < 0.0001$] and WC [−3.0 (3.8) cm; $P < 0.0001$] to a greater extent than CONTROL [weight: −0.4 (3.1) kg; $P = 0.51$; WC: −1.4 (4.5) cm; $P = 0.02$]. In CONTROL, 7% reduced ≥ 5% weight and 31% reduced ≥ 10 mmHg SBP, whereas, in the NUT group, 18% reduced ≥ 5% weight and 47% reduced ≥ 10 mmHg SBP. Patients in NUT (versus CONTROL) were more likely to lose ≥ 5% of weight (odds ratio = 4.27, 95% confidence interval = 1.69–10.80; $P < 0.01$) and reduce SBP ≥ 10 mmHg (odds ratio = 3.15, 95% confidence interval = 1.58–6.27; $P < 0.01$).

Conclusions: Patients who received nutritional counselling during CR improved anthropometric measures and were more likely to lose weight and reduce SBP than patients without nutritional counselling.

Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide, which accounts for 31% of total deaths [(1)]. A high incidence of CVD along with high survival rates (as a result of adequate treatment access) may decrease quality of life years (QALY) and increase disability-adjusted life years (DALY) of the Chilean population [(1,2)]. Primary and secondary prevention is then essential to reduce CVD risk considering that high systolic blood pressure (SBP), high low-density lipoprotein (LDL)-cholesterol, high glycaemia, obesity, and low diet quality are the top five risk factors for

global mortality and QALY loss [1]. Cardiac rehabilitation (CR) is a class IA recommendation for coronary heart disease in the American guidelines. CR has shown to reduce cardiovascular mortality by 26–30%, cardiovascular events by 43% and the hospitalisation rate in coronary patients by 18–26%. CR programmes including exercise also achieve better outcomes [3,4,5]. Despite these benefits and its cost-effectiveness, attendance to CR is usually low [6,7]. In the USA and England, between 14% and 37% of eligible patients attended CR, whereas only 27% completed it [7,8]. In Chile, approximately 5% of patients attended a CR programme after acute myocardial infarction (AMI) and only one-third of them completed the programme [9,10]. Furthermore, a low proportion of them achieve SBP, LDL-c-holesterol, weight loss or physical activity goals after 1 year of AMI [11–12].

In Chile, there is 0.5 CR per 1 million inhabitants, whereas it is widely implemented in the USA, Canada, Australia and most European countries [13]. Core components of CR programmes include the management of CV risk factors (blood pressure, cholesterol and diabetes), weight management, nutrition counselling, tobacco cessation, psychosocial management and exercise training, which together reduce CV risk and help improve patient outcomes and quality of life [14]. CR programmes with a dietitian in their team report a higher number of nutrition services, including group sessions and one-on-one counselling, which might improve patient outcomes [15]. Overall, nutritional intervention in CR programmes reports a reduction of body weight, waist circumference (WC), LDL-cholesterol, triglycerides and an increase in diet scores and patients' self-efficacy [16–20].

Few studies have compared anthropometric and biochemical outcomes of patients receiving nutritional counselling when attending a CR programme. Also, there is no study comparing blood pressure (BP) outcomes of CR patients. Holmes *et al.*[16] reported that only patients with dietitian intervention (versus without nutritional counselling) reduced LDL-cholesterol, triglycerides, waist circumference and body mass index (BMI). It should be considered that control patients had both lower BMI and waist circumference, which can affect the results, because the changes in anthropometric variables are also dependent on initial body weight. Regarding BP, only Riegel *et al.* [21] compared the outcomes between interventions delivered by a dietitian versus other health professionals, finding that SBP is reduced with the dietitian intervention, but not by the other interventions. Unfortunately, they did not report outcomes in patients attending a CR programme or in secondary prevention. The present study compared changes in weight, WC and BP of patients attending CR with (NUT) and without (CONTROL) nutritional intervention.

Materials and methods

Patients

We collected demographic data [age, sex, medical history and cardiovascular risk factors (RFs)], anthropometric measurements (weight, WC and BMI) and BP from patients who attended CR between 2003 and 2019 in an ambulatory setting of a university hospital. All patients provided their written informed consent, authorising the collection of their data anonymously for academic purposes.

Design

The design of the study was retrospective and analytical. Inclusion criteria were patients who completed 36 sessions of a phase II CR programme. We excluded patients <18 years old, with a BMI < 20 kg m^{-2}, and those with a missing value for body weight (baseline or 36 sessions). Three-hundred and eight patients completed CR between 2003 and 2019, whereas 272 (88%) met the inclusion criteria. Before 2010, the CR team did not include a dietitian; therefore, patients were divided into two groups: (i) CONTROL, without nutritional intervention (attended CR between 2003 and 2009) and (ii) NUT, subjects with at least two appointments with the dietitian (attended CR between 2010 and 2019). Both groups included patients who attended for primary (without previous CV events, but high cardiovascular risk) and secondary prevention (with a previous CV event).

Cardiac rehabilitation programme

For admission, patients had a cardiac stress test besides being referred by a cardiologist. Patients included in the analysis completed the 36 sessions of the CR programme, with two to five sessions per week. Before each session, a nurse controlled their vital signs at rest (heart rate and BP). During the session, patients completed 30–40 min of aerobic exercise in a treadmill or bicycle at 50–70% of their maximal capacity (determined by the maximum metabolic equivalent tasks and heart rate achieved in the cardiac stress test). Heart rate, BP and perceived exertion (determined by a Borg scale) were controlled during exercise. Patients also performed resistance and flexibility exercises during each session.

Nutritional counselling

Patients with nutritional intervention attended a monthly appointment with the dietitian of the programme for assessment and counselling: at admission, as well as at the

18th and 36th sessions. The dietitian measured weight, waist circumference and skinfolds to estimate mid-arm muscle circumference and body fat. The baseline diet was evaluated using 24-h recall and a screener for Mediterranean diet adherence [22]. Goals were agreed with each patient according to initial nutritional status, body composition, dietary habits and medical history. An individualised diet plan was delivered to the patients according to energy and macronutrient requirements to meet their goals. Overall, diets were planned with 1–1.2 g kg^{-1} of protein. Energy restriction between 500 and 1000 kcal day^{-1} was also recommended to overweight and obese subjects (energy restrictions were adjusted to ensure a total caloric intake ≥ resting energy expenditure). A Mediterranean dietary pattern was emphasised with a reduction of saturated fat and sodium intake in all diet plans. Other counselling or assessment appointments could be arranged if the patient required it.

Measurements and procedures

All subjects who attended the CR programme underwent a baseline and final evaluation (at session 36). The CR nurse (2003–2009) or dietitian (2010–2019) measured weight and height using a standard scale with a capacity of 220 kg (model 700; Seca GmbH, Hamburg, Germany) to a precision of 100 g and 0.1 cm, respectively. Nutritional status was classified according to the World Health Organization (WHO) criteria: normal weight individuals were those with a BMI between 20 and <25 kg/m^2; overweight individuals were those with a BMI ≥25 kg/m^2 and <30 kg/m^2, and obese individuals were those with a BMI ≥30 kg/m^2. WC was measured according to WHO criteria: on the midpoint between the last rib and the iliac crest. Optimal WC was defined as <80 cm for women and <90 cm for men, according to the harmonised ATP III criteria [23]. Subjects performed a 6-min walk test (6 MWT) at the first and final session of the programme; the nurse measured BP after a 5-min rest, with the patient seated before the test. The 6 MWT is a submaximal test of functional capacity, in which the total distance walked correlates with maximal aerobic capacity [24].

Statistical analysis

Baseline characteristics of both groups were compared using an independent sample *t*-test and Fisher;s exact test. Repeated-measures analysis of variance (ANOVA) with a Tukey–Kramer post-hoc test was performed to compare weight, waist, BMI, 6 MWT distance, SBP and diastolic blood pressure (DBP) differences between groups (NUT versus control) and time (baseline versus session 36). An analysis of covariance was performed to adjust the group's effect over the dependent variables (changes of weight, BMI, WC, 6 MWT distance and BP). Covariates considered in this model were the respective baseline values and variables that differed between both groups (e.g. sex, age groups, risk factor aggregation, treatment and anti-hypertensive medication). Covariables that had a significant effect were also assessed for interaction with the group's effect. If the interaction was not significant, we interpreted the group and covariables effect independently.

We performed a two-way ANOVA with Sidak's post-hoc test to determine whether other variables affected weight loss and interacted with the intervention as a possible source of bias. The variables included in this analysis were: age (<50 years, 50–64 years old and ≥65 years old), sex, nutritional status, season at admission (winter versus summer), chronic diseases diagnosis, type of treatment (surgery, angioplasty only and non-invasive) and type of prevention (primary versus secondary). Achievement of the following goals was also determined: BMI <25 kg/m^2, BMI <30 kg/m^2, SBP <130 mmHg, DBP <80 mmHg, weight loss ≥5% and SBP reduction ≥10 mmHg. The probability of reducing at least 5% of the initial weight and 10 mmHg of SBP was determined using logistic regression models adjusted by age, sex and initial BMI. A second model for SBP reduction was also adjusted by baseline SBP and anti-hypertensive medication. For the analyses, we used PRISM, version 8.4.2 (GraphPad Software Inc., San Diego, CA, USA) and SPSS, version 17.0 (SPSS Inc., Chicago, IL, USA).

Results

For the analysis, 272 patients had data at baseline and session 36 for body weight, 270 for BMI, 241 for BP and 209 for WC. Table 1 shows the baseline characteristics of CONTROL and NUT. Both groups had similar age, BMI, WC and prevalence of cardiovascular risk factors. Obesity rate was 28% and 30% in CONTROL ($n = 40$) and NUT ($n = 38$), respectively. Compared with the CONTROL, NUT had a higher proportion of women (27% versus 17%, respectively; $P < 0.05$), older adults (40% versus 23%, respectively; $P < 0.01$), patients with angioplasty (42% versus 27%, respectively; $P < 0.05$), and patients with ≥2 RFs aggregation (69% versus 57%, respectively; $P = 0.05$). Time for completion of the 36 sessions varied for each individual, with a mean (SD) follow-up of 4.6 (1.6) months in CONTROL and 4.2 (1.8) months in NUT ($P = 0.054$). Patients in NUT attended to a mean (SD) of 3 (1) dietitian appointments during CR, and more patients reduced ≥5% of initial body weight (18% versus 7%; $P < 0.01$) and ≥10 mmHg SBP (47% versus 31%; $P = 0.01$) compared to CONTROL.

Table 1 Demographics, anthropometrical data and medical history of patients completing cardiac rehabilitation with (NUT) and without nutritional counselling (CONTROL)

Demographical, medical and anthropometrical variables	CONTROL (n = 144)	NUT (n = 128)	P-value
Age (years)	59 (12)	60 (13)	0.64
≥65 years (%)	23%	40%	<0.01
Women (%)	17%	27%	0.05
Primary prevention (%)	35%	26%	0.10
Intervention during spring or summer (%)	51%	47%	0.54
Anthropometric measurements			
BMI (kg m^{-2})	28 (3.9)	28 (4.0)	0.99
Weight (kg)	80.1 (11.5)	78.5 (13.0)	0.28
Waist circumference (cm)	97 (10)	97 (12)	0.97
Weight loss ≥ 5% (%)	7%	18%	<0.01
Weight loss $month^{-1}$ [initial weight]	−0.1 (0.8)	−0.4 (1.2)	0.02
Risk factors			
Overweight and obesity (%)	78%	77%	0.88
Systolic BP (mmHg)	114 (16)	110 (14)	0.14
Diastolic BP (mmHg)	68 (8)	67 (8)	0.50
SBP reduction ≥ 10 mmHg (%)	31%	47%	0.01
6 MWT distance[a] (m)	537 (94)	520 (86)	0.11
Dyslipidemia (%)	76%	82%	0.23
Hypertension (%)	64%	74%	0.09
Diabetes (%)	15%	14%	1.0
Smoking (%)	5%	7%	0.46
≥2 risk factors (%)	57%	69%	0.05
Tobacco cessation (%)	51%	44%	0.35
Acute myocardial infarction (%)	47%	54%	0.36
Heart failure/other cardiopathies (%)	5%	8%	0.29
Cerebrovascular event (%)	4%	2%	0.45
Medical intervention			
Cardiac transplant (%)	1%	3%	0.42
Surgery[c,d] (%)	26%	25%	0.78
Angioplasty/stent[d] (%)	27%	42%	0.01

Data are expressed as the mean (SD) or percentage when indicated for prevalence. The differences between groups were determined by an independent sample Student's t-test for continuous variables and Fisher's exact test for categorical variables.

BMI, body mass index; 6 MWT, six-minute walk test; BP, blood pressure; CONTROL, without nutritional intervention; NUT, with nutritional intervention.

[a]Walked distance in 6 MWT is a submaximal measurement of aerobic capacity.

[b]For ≥ 2 risk factor aggregation, we considered the following risk factors: smoking, diabetes, hypertension and dyslipidemia.

[c]Includes patients with coronary artery bypass grafting and heart valve repair or replacement.

[d]Patients with angioplasty and surgery history were included in both groups.

Table 2 shows weight, BMI, WC, BP and 6 MWT distance at baseline and after 36 sessions of CR. Both groups had similar baseline characteristics, although the change of anthropometric parameters was greater in NUT compared to CONTROL. Also, NUT had a greater mean (SD) increase in 6 MWT distance than CONTROL [71 (50) m versus 55 (48) m, respectively; P = 0.02]. Figure 1 shows the mean changes and 95% confidence intervals (CIs) for each variable in both groups, as well as differences within and between groups (group × time interaction). A group × time interaction was observed in all anthropometric parameters, whereas SBP and DBP only had a time effect ($P < 0.05$) with no differences between groups. CONTROL only had a mean reduction of WC (−1.4 cm; 95% CI = −2.3 to −0.4); whereas NUT had a higher mean reduction of all anthropometric parameters: WC (−3.0 cm; 95% CI = −3.6 to −2.4), weight (−1.3 kg; 95% CI = −1.8 to −0.7) and BMI (−0.4 kg/m^2; 95% CI = −0.6 to −0.2).

Weight, BMI, WC, 6 MWT distance, SBP and DBP changes were inversely associated with their baseline values ($P < 0.0001$). The covariance analysis adjusting the group's effect by baseline values and including variables that differed between groups (angioplasty, sex, age ≥65 years, RFs ≥2, anti-hypertensive medication) only showed that sex was a significant covariable for changes

Table 2 Anthropometric parameters, blood pressure and six-minute walk distance at baseline and 36 sessions of cardiac rehabilitation patients with and without nutritional intervention

Variable	CONTROL (2003–2009; n = 144)			NUT (2010–2019; n = 128)		
	Baseline	36 sessions	*P*-value	Baseline	36 sessions	*P*-value
Weight (kg)	80.1 (11.5)	79.7 (10.6)	0.51	78.5 (13.0)	77.2 (12.3)	<0.0001
BMI (kg m^{-2})	28.0 (3.9)	27.9 (3.6)	0.51	28.0 (4.0)	27.6 (3.8)	<0.0001
Waist circumference (cm)	97.1 (10.2)	95.7 (9.9)	0.02	97.0 (12.3)	94.0 (11.4)	<0.0001
SBP (mmHg)	114 (16)	112 (15)	0.66	110 (14)	107 (13)	0.18
DBP (mmHg)	68 (8)	67 (8)	0.37	67 (8)	65 (9)	0.13
6 MWT distance (m)	537 (94)	592 (92)	<0.0001	520 (86)	591 (89)	<0.0001

Mean values (baseline versus 36 sessions) were compared using repeated-measures analysis of variance adjusted by the Tukey–Kramer post-hoc test in both groups. BMI, body mass index; 6 MWT, six-minute walk test; DBP, diastolic blood pressure; CONTROL, without nutritional intervention; NUT, with nutritional intervention; SBP, systolic blood pressure.

in weight ($P < 0.0001$), WC ($P < 0.01$), BMI ($P = 0.01$) and 6 MWT distance ($P = 0.02$). However, we did not find interaction between group and sex (weight: $P = 0.24$; BMI: $P = 0.23$; WC: $P = 0.39$; 6 MWT distance: $P = 0.67$). Changes remained higher in NUT versus CONTROL for weight ($P = 0.01$), BMI ($P = 0.02$), WC ($P < 0.01$), 6 MWT distance ($P < 0.01$) and SBP ($P = 0.04$) in the adjusted the models.

We determined the normalised percentage of weight loss by time once completed 36 sessions in both groups (% weight/month). Relative mean (SD) reduction per month was higher in NUT versus CONTROL [−0.4% (1.2%) versus −0.1% (0.8%) weight/month, respectively; $P = 0.02$).

A sub-analysis that included only overweight and obese patients (BMI ≥25 kg/m^2; $n = 210$) also showed that NUT ($n = 99$) had a greater reduction of weight (−1.7 versus −0.6 kg; $P = 0.02$), BMI (−0.6 versus −0.2 kg/m^2; $P = 0.02$) and WC (−3.5 versus −1.4 cm; $P < 0.0001$) and that SBP reduction did not significantly differ between NUT and CONTROL (−4.1 versus −2.4 mmHg; $P = 0.46$). More patients in NUT (BMI ≥25 kg/m^2) lost more than 5% weight compared to CONTROL (21% versus 7%, respectively; $P = 0.002$). This difference remained when only obese patients ($n = 78$) were included (29% versus 10%, respectively; $P = 0.04$).

Table 3 summarises weight loss among different subgroups. Overall, they did not interact with the nutritional intervention's effect, although we observed a trend for interaction with treatment ($P = 0.07$). Sex, nutritional status, diabetes, type of treatment and primary prevention affected weight loss. Also, we observed a trend for age.

Adjusted multivariate models showed that patients in NUT were more likely to reduce ≥5% of the initial body weight (odds ratio = 4.27, 95% CI = 1.69–10.80; $P < 0.01$) and ≥10 mmHg in SBP (odds ratio = 3.15, 95% CI = 1.58–6.27; $P < 0.01$) (Table 4). There were no differences in the achievement of optimal anthropometric and BP goals between groups during the intervention (Table 5).

Discussion

The results obtained in the present study show that a dietitian-delivered intervention decreases weight, BMI, waist circumference and SBP of patients attending a CR programme. They were at least four-fold more likely to lose at least 5% of initial body weight, considering that not all CR patients were overweight and that weight loss is a secondary outcome of the programme. Also, NUT patients were three-fold more likely to reduce SBP by 10 mmHg compared to CONTROL.

Gomadam *et al.* [19] reported that, on average, patients do not lose significant weight during CR. Similar to our results, they reported that 14% of their patients lost ≥5% of initial weight, being higher in obese patients. Lavie *et al.* [20] also reported that 36% of obese patients in CR who attended a monthly dietitian appointment reduced significant body weight and had greater improvements in biochemical parameters than those who fail to lose weight. However, in contrast to the present study, they did not compare the intervention with a control group, and they did not describe the nutritional intervention. We found a higher proportion of patients with significant weight loss when a dietitian delivered the intervention than CR alone (18% versus 7%). Overall, patients with nutritional intervention lost about 1 kg and 2 cm more of weight and waist circumference, respectively, than controls. Prior *et al.* [18] also reported similar reductions of weight, BMI, waist, and SBP (1.4 kg, 0.5 kg m^{-2}, 2.6 cm, and 3 mmHg, respectively), although they did not compare these outcomes with a control group. Holmes *et al.* [16] reported a modest reduction of BMI after CR when they included a dietitian-delivered intervention. Although

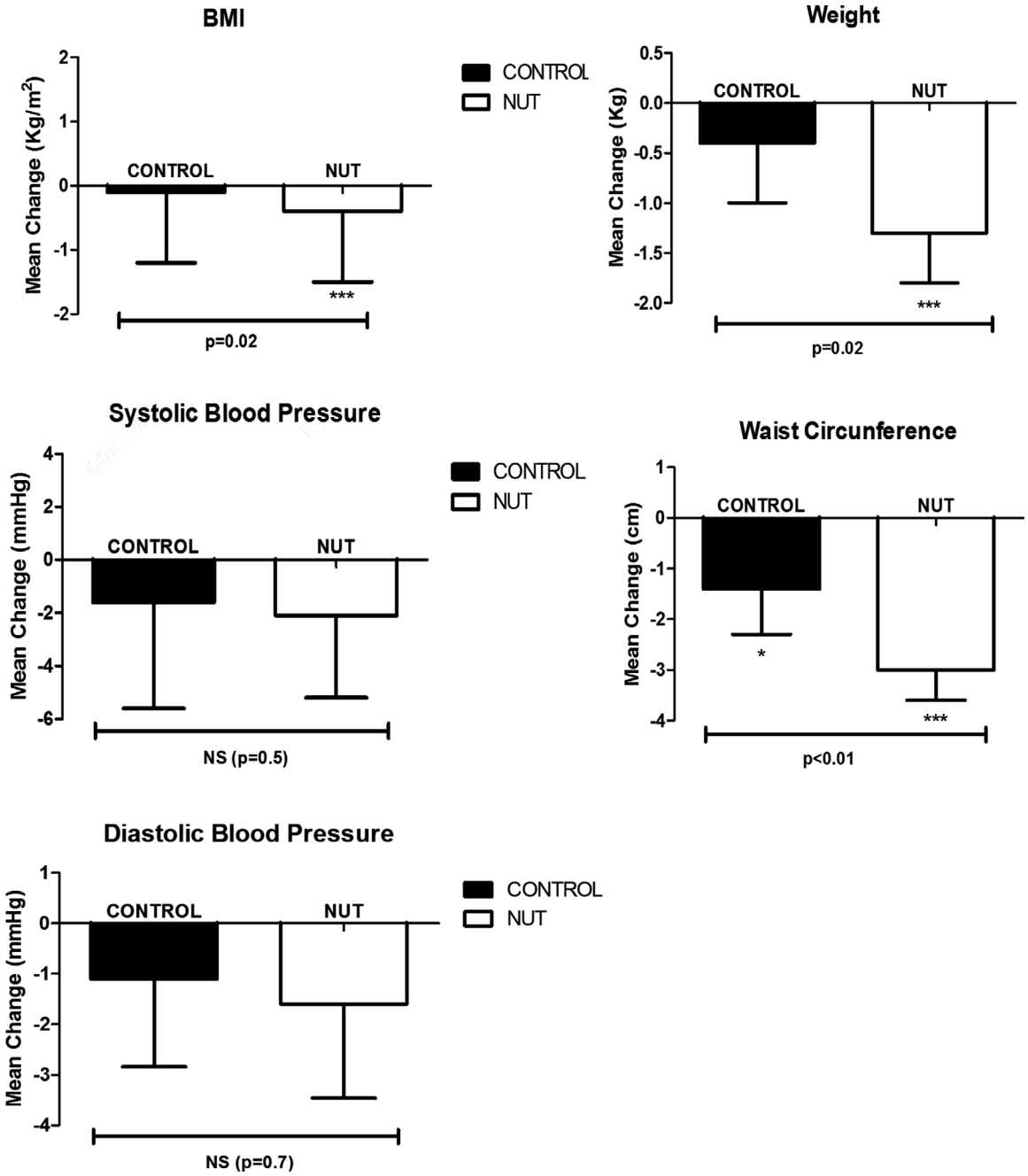

Figure 1 Change (36th session – baseline measurement) of weight, body mass index (BMI), waist circumference, and blood pressure of patients with and without nutritional intervention during cardiac rehabilitation. Bars represent the mean change (36th session – baseline measurement) with 95% confidence interval (error bars) of anthropometric parameters and blood pressure during cardiac rehabilitation. CONTROL (black bars): group without nutritional intervention. NUT (white bars): group with nutritional intervention. Differences between groups were determined using repeated-measures analysis of variance with a Tukey–Kramer post-hoc test. *P*-values indicate differences between both groups (group × time interaction). $*P < 0.05$; $***P < 0.001$ for differences between baseline and 36 session measurement within each group according to a post-hoc test. NS, not significant.

the baseline characteristics of their patients (such as age, the proportion of women and waist circumference) were similar to ours, patients in the present study doubled the reduction of waist circumference reported by them [16]. Other studies have reported higher weight loss (−5.2 kg) after 4 months of a weight management intervention in

Table 3 Mean weight change according to subgroups of age, sex, nutritional status, the season of intervention, chronic diseases, type of prevention, and treatment of patients with and without nutritional intervention during cardiac rehabilitation

Subgroups	Intervention Group				
	CONTROL, Weight change, kg (n = 144)	NUT, Weight change, kg (n = 128)	Sub-group (S)	Nutritional Intervention (I)	S × I (P-value)
Age group					
Age < 50 years	−1.0 ± 4.0 (24)	−2.2 ± 3.8 (23)	0.09	0.02	0.90
Age 50–64 years	−0.4 ± 3.2 (87)	−1.2 ± 3.3 (54)			
Age ≥ 65 years	0.1 ± 1.8 (33)	−0.9 ± 2.5 (51)			
Sex					
Men	−0.2 ± 3.1 (120)	−0.9 ± 3.1 (94)	0.01	0.07	0.82
Women	−1.2 ± 2.9 (24)	−2.2 ± 3.2 (34)			
Nutritional status					
Obese	−2.0 ± 4.5[a] (40)	−2.5 ± 3.5[a] (37)	<0.001	0.05	0.50
Overweight	0.2 ± 2.3[b] (71)	−1.2 ± 2.9* (62)			
Normal weight	0.5 ± 1.7[b] (31)	0.1 ± 2.6[b] (29)			
Season at admission					
Intervention at spring or summer	−0.04 ± 2.8 (73)	−1.3 ± 4.5 (60)	0.30	0.01	0.67
Intervention at fall or winter	−0.67 ± 3.4 (71)	−1.6 ± 3.3 (68)			
Chronic diseases					
Hypertensive	−0.4 ± 3.6 (92)	−1.5 ± 4.0* (94)	0.21	0.09	0.36
Normotensive	−0.3 ± 2.5 (51)	−0.6 ± 3.6 (33)			
Diabetic	1.4 ± 2.9[a] (21)	0.2 ± 2.2 (18)	0.009	0.02	0.41
Non-diabetic	−0.6 ± 3.1[b] (121)	−1.4 ± 3.2 (110)			
Prevention					
Secondary Prevention	0.3 ± 2.5[a] (93)	−0.9 ± 3.2* (95)	0.0002	0.02	0.50
Primary prevention	−1.5 ± 3.6[b] (51)	−2.2 ± 2.9 (33)			
Type of Treatment					
Invasive, Surgery or Transplant	0.7 ± 2.6[a] (38)	−0.6 ± 3.0 (36)	0.04	0.005	0.07
Minimally invasive, Angioplasty/ STENT only	0.3 ± 1.8 (30)	−1.9 ± 3.2* (46)			
Non-invasive, Medical Therapy	−1.1 ± 3.5[b] (75)	−1.1 ± 3.2 (44)			

Data are expressed as mean ± standard deviation. CONTROL: without nutritional intervention, NUT: with nutritional intervention. Means were compared using Two-way analysis of variance (ANOVA) with the intervention (I), subgroups (S), and their interaction (S × I) as factors. Multiple comparisons were adjusted by Sidak *post-hoc* test. Values with different superscript letters in each column are significantly different. Subgroups: age, sex, nutritional status, season, chronic diseases, prevention, and treatment. Intervention groups: NUT and Control. If the *P-value* for interaction > 0.05, the intervention's effect on weight loss is independent of the respective subgroups' effect.
*P < 0.05 for the difference with respective subgroup in the control group.

Table 4 Probability of reducing weight (≥ 5%) and systolic blood pressure (≥10 mmHg) with nutritional intervention during a cardiac rehabilitation program

	Unadjusted Models		Model 1		Model 2	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Weight loss ≥ 5%	2.91 (1.34–6.29)	0.01	4.27 (1.69–10.80)	0.002	–	–
SBP reduction ≥ 10 mmHg	1.96 (1.16–3.31)	0.02	2.31 (1.29–4.14)	0.005	3.15 (1.58–6.27)	0.001

The probability of reducing weight and blood pressure was determined using unadjusted and adjusted logistic regression models. OR, odds ratio; 95% CI, 95% confidence interval; SBP, systolic blood pressure. Model 1: Adjusted by age, sex and baseline body mass index (BMI). Model 2: Adjusted by age, sex, baseline BMI, baseline SBP and anti-hypertensive medication. Reference (OR = 1): Cardiac rehabilitation without nutritional intervention (CONTROL).

obese patients attending to CR [25]. Nevertheless, the latter study was both an early intervention (2 weeks after discharge from hospital) and conducted in patients with a higher BMI than ours (mean BMI = 37.6 versus 28 kg m^{-2}, respectively), which may explain the differences compared to our results.

Table 5 Anthropometric and blood pressure outcomes according to established goals in patients attending cardiac rehabilitation with (NUT) or without nutritional intervention (CONTROL)

	CONTROL (2003–2009)			NUT (2010–2019)		
Variable	Baseline	36 sessions	*P*-value	Baseline	36 sessions	*P*-value
BMI <25 kg m^{-2} (%)	22% *n* = 142	23%	NS	23% *n* = 128	27%	NS
BMI <30 kg m^{-2} (%)	73% *n* = 142	78%	NS	70% *n* = 128	77%	NS
Waist circumference <80/90 cm (%)	19% *n* = 83	20%	NS	18% *n* = 128	23%	NS
SBP <130 mmHg (%)	78% *n* = 123	83%	NS	88% *n* = 120	92%	NS
DBP, <80 mmHg (%)	76% *n* = 123	85%	NS	85% *n* = 120	87%	NS

BMI, body mass index; CONTROL, Without nutritional intervention; DBP, diastolic blood pressure; NUT, With nutritional intervention; SBP, systolic blood pressure. Waist circumference goal was < 80 cm in women and < 90 cm in men. Intragroup and Intergroup differences were evaluated using Fisher exact test. NS, not significant.

A meta-analysis that evaluated the effect of a dietitian in traditional weight management interventions reported an additional weight loss of 1.0 kg (0.4 kg m^{-2}), which aligns with our findings in CR patients [26]. Most of the studies evaluating traditional weight management in obese patients reported a short-term weight loss between 1.6 and 2.5 kg after the intervention (6–12 months). Although this weight change may not be considered clinically relevant, most of these studies reported improvements in lipid profile, BP and glycaemia after the intervention even when they did not achieve weight goals [16,20,27–33]. Diet quality modification and physical activity interventions, rather than a significant reduction of caloric intake, may drive these improvements.

Aerobic training during CR improves functional capacity and CV risk factors, including BP. In the present study, both groups improved the 6-min walk distance to a clinically relevant extent (≥30.5 m), whereas they also reduced SBP by approximately 2 mmHg, with a time effect, which accounts for the effect of CR alone [34]. Although we found a mild reduction of SBP, some important randomised clinical trials have reported a reduction between 1 and 3 mmHg of SBP using a drug, such as ramipril in the HOPE study, showing a significant reduction of CV risk (13–24%) [35]. We also found that patients in NUT had a higher probability of reducing SBP ≥10 mmHg than the control group; such SBP reduction decreases the CV risk by 20% [36]. A higher improvement of physical function determined by 6 MWT in NUT can also help to explain better SBP outcomes. However, we did not find any correlation between SBP and 6 MWT distance variables (data not shown) and all patients received the same exercise intervention based on their initial aerobic capacity. In the present study, we cannot determine causality or whether a higher weight loss with NUT intervention or other variables (e.g. self-efficacy) can affect 6 MWT outcomes, although some studies have reported improved aerobic capacity with weight loss and dietary intervention [20,37]. Lavie *et al.* [20] reported a better improvement in aerobic capacity, as estimated by metabolic equivalent tasks, in CR patients who lost significant weight. Moreover, in obese heart failure patients, Kitzman *et al.* [37] found that aerobic capacity, as measured by either peak VO_2 or 6 MWT, improved independently with diet intervention and that the effect was additive to exercise.

Twenty-nine percent of our obese patients with nutritional intervention lost ≥5% weight, which is better than the rate reported by Delahanty *et al.* [31] for one-on-one dietitian counselling (21%), but worse than intensive interventions that include group sessions (46%). [38] CR without a dietitian-delivered intervention still had a lower weight loss success in our obese patients (10%). Predictors of successful weight loss are initial weight loss >10% in 6 months, use of meal replacement, regular physical activity of 287 min $week^{-1}$ and group sessions (19 in six months) [28,31,38,39]. We did not directly apply these strategies in our intervention programme, although CR includes physical activity and self-care group education.

Practice guidelines for nutrition recommend an initial assessment with two follow-up appointments during the first 6 weeks, which has shown a superior effect on glycaemia and weight loss in diabetic patients than usual care (one appointment with the dietitian) [40-42]. We standardised three dietitian appointments during the 36 sessions of CR (3–4 months), although some overweight patients attended to additional appointments for weight management. More aggressive approaches, such as frequent group sessions, might improve adherence and outcomes of CR patients. Our results show that reinforcing the dietitian intervention increases the probability of achieving a clinically relevant reduction in weight and SBP, which further reduces CV risk after CR.

Age, sex and BMI are known factors affecting energy expenditure and weight loss [27,43]. However, it is surprising that women, primary prevention patients and non-diabetics lost more weight than their counterparts [44]. A gender gap for weight perception and intention for

weight loss, especially in overweight women, might explain this difference during CR [45]. Furthermore, we expected that patients in secondary prevention, comprising those who had a history of CV events, would have better outcomes than those in primary prevention (higher disease awareness), whereas, surprisingly, they lost less weight. Usually, primary prevention patients are more resistant to change, although those completing CR might be the exception. Also, a greater weight loss previous to CR admission (e.g. during the hospitalisation after bypass surgery or percutaneous coronary interventions) can explain these results. In this regard, DiMaria-Ghalili *et al.* [46] reported that 86% of patients lost an average of 3.7 kg and reduced usual physical activity within one month after cardiac surgery. Therefore, many patients in secondary prevention may recover some weight during CR, primarily from muscle mass. The latter could explain the trend for interaction between nutritional intervention and treatment (weight loss was higher in NUT mainly in patients with angioplasty and surgery), suggesting that nutritional intervention improves weight control after invasive treatment. Further research with usual weight and body composition measurements is warranted.

The present study has limitations, and the results should be interpreted carefully, especially for BP data. Initial and final BP was collected only once at admission and on completion of CR. Second, medication guidelines and effectiveness might change over 10 years (time difference between the two study groups), which might affect BP outcomes. Therefore, we included drug therapy for hypertension in the covariance analysis; also, both groups had similar baseline BPs and medications rarely change during CR. Third, the retrospective design of the study cannot guarantee a standard nutritional intervention and follow-up time for all the patients. However, the mean difference between groups for completing the 36 sessions was less than 15 days (<0.5 months), which may not affect our results. We also adjusted the percentage of weight loss by time for completing the 36 sessions (% weight loss/month) and obtained similar results. Finally, we included patients who had a healthy body weight in the analysis, which may underestimate the outcomes for a more overweight population, which is the reason why we also adjusted by initial BMI, and performed an additional analysis in this subgroup. We must highlight that the inclusion of a similar control group strengthens our analysis and results.

In conclusion, nutritional intervention delivered by a dietitian during CR has modest but significant effects on patient weight, waist and BMI, and it also increases the odds of significantly losing weight and blood pressure compared to the control group. 6 MWT distance also improved to a greater extent with nutritional intervention; however, more research is required to clarify causality. Including a dietitian in the CR team improves patient outcomes and should be encouraged in all secondary prevention and CR programmes.

Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.
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All authors critically reviewed the manuscript and approved the final version submitted for publication. GV and MA drafted the first manuscript. GV, JG, and MA3 contributed in the conception design and analysis. LO, MA2 and AB collected the data. All authors reviewed and commented in subsequent drafts of the manuscript.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with STROBE guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

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CARDIOVASCULAR DISEASE

Observational study evaluating the nutritional impact of changing from 1% to 2% propofol in a cardiothoracic adult critical care unit

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Keywords
critical illness, enteral nutrition, non-nutritional energy, overfeeding, propofol, underfeeding.

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Abstract

Background: Nutritional support in the critically ill aims to avoid under and overfeeding, adjusting to changes in energy expenditure during critical illness. The sedation propofol provides significant fat and energy load. We investigated whether changing from 1% to a 2% propofol, would decrease non-nutritional energy, avoid energy overfeeding and increase the amount of protein delivered.
Methods: A retrospective observational study was performed. The primary outcome was protein delivery. Secondary outcomes were energy from propofol fat and the total energy delivered from nutrition and propofol.
Results: In total, 100 patients were investigated, with 50 patients in each group. The propofol dose was comparable for each group. The nutrition energy prescribed was significantly less for the 1% compared to 2% group, taking the energy from propofol into consideration. Both groups had similar protein targets, although the amount delivered was significantly higher in the 2% group. Thirty-six percent of individuals receiving 1% exceeded 45% of total energy from fat. The poor delivery of nutrition resulted in inadequate energy and protein, irrespective of propofol dose.
Conclusions: We investigated the impact of propofol on energy overfeeding and under delivery of protein, and highlighted suboptimal nutritional provision. Work is needed to investigate the harm that high-fat delivery may pose in light of poor nutrition delivery.

Introduction

The aim of nutritional support in critically ill patients is to achieve energy balance (avoiding under and overfeeding) and provide sufficient protein to meet macro- and micronutrient requirements, as well as maintain gut integrity. These interventions support the immune system and reduce the frequency of hospital-acquired infections [1,2]. Energy expenditure may change during the course of critical illness and therefore this requires regular review. Exceeding the energy requirements may be particularly deleterious during the early phase of acute illness as a result of endogenous substrate mobilisation via lipolysis and gluconeogensis [3]. Overfeeding is associated with many complications, such as hyperglycaemia, hepatic steatosis, infectious morbidity, increased length of stay and even mortality [4].

In addition to the provision of enteral and parenteral nutrition, critically ill patients can receive other non-nutritional sources of energy, particularly from the sedative agent propofol. It comes in a lipid solution containing between 1.035–1.1 kcal mL^{-1} and 0.1 g of fat per mL (depending on the manufacturer) and can provide a significant fat and energy load [5,6]. The latest European Society for Enteral and Parenteral Nutrition guidelines (ESPEN) guidelines [4] recommend monitoring energy

intake from propofol and adjusting nutritional plans to avoid excess fat and risk of harmful overfeeding. However, because enteral nutrition (EN) products have fixed energy and protein ratios, reducing EN energy delivered also reduces protein, carbohydrate, vitamins and minerals [7]. Providing adequate protein during critical illness is considered to help prevent loss of muscle mass and help restore it during recovery [8]. It is therefore imperative to find ways to optimise protein delivery when using the energy dense propofol solution.

Taylor *et al.* [7] proposed the substitution of 2% for 1% propofol as a potential solution because 2% provides the same dose of sedative but in half the volume and therefore half the fat load. This study was hypothesis generating and, to date, it is still not known whether making this switch in a clinical setting has a positive impact on protein delivery. Following on from this prior work, we set out to address the hypothesis that changing from a 1% to 2% propofol solution would decrease non-nutrition energy and increase the amount of protein delivered. We did this by conducting a retrospective pre- and post-observational study.

Materials and methods

Study design

Recruitment took place on an adult cardiothoracic intensive care unit (ICU) in London and included participants aged between 16 and 85 years. The primary objective of the study was to examine the effect of changing from 1% to 2% propofol on protein delivery. Secondary outcomes were the daily contribution of non-nutritional energy derived from propofol fat and the total nutritional energy delivered from the combination of enteral or parenteral nutrition and propofol.

All participants were included if they were in the ICU for longer than 72 h, received propofol more for at least 3 days and received enteral and/or parenteral feeding. Exclusion criteria were self-ventilating patients, those mechanically ventilated on propofol for less than 72 h and those not receiving nutrition support. We only included those with an ICU stay longer than 3 days because we considered that the impacts of under and over feeding become more apparent with longer ICU stays. This was a retrospective pre–post observational study. Patients were recruited who met the inclusion criteria during the two data collection periods, each lasting 3 months. Fifty patients were selected over the 1% phase between February and April 2019 and a different 50 patients once the 2% propofol had been implemented, from May to July 2019. The propofol used was Lipuro (Bbraun, Melsungen, Germany) which contains 1.035 kcal mL^{-1}. There was no merging of usage when we switched from 1% to 2%. Any remaining 1% propofol was removed from the ICU once the change over occurred, and so no patients in the 2% group received any 1% solution.

Nutritional intake

Patients were fed according to the ICU feeding protocol, which stated that EN should be the first choice, and commenced within 48 h of admission. If EN failed or was contraindicated, parenteral nutrition (PN) was commenced. The treating dietitian set the energy, protein and fat targets in accordance with the ESPEN guidelines [4]. These comprised: energy 20–25 kcal kg^{-1} (using an ideal or adjusted weight for those body mass index (BMI) >30); 1.3 g protein kg^{-1} and 1.5 g kg^{-1} fat. Nutritional intake data were collected on a daily basis for the 7 days that the patient remained in the study. We are aware that the amount of nutrition support delivered would be a confounder, and so we encouraged strict adherence to our unit fasting guidelines.

For the analysis, the energy, protein and fat prescription and amount received are expressed as kcal kg^{-1} and g kg^{-1}, respectively. This is the overall intake divided by the body weight, allowing for direct comparisons with other studies. Adequacy of nutrition delivery was defined as 80% or more for energy and protein [9,10]. For each patient, the proportion of energy intake from fat was calculated. High-fat delivery was defined to occur when >45% of total energy was supplied from fat [5].

The project was submitted to and approved by HRA and Health and Care Research Wales (HCRW). IRAS reference number: 286229, REC reference 20/HRA/3491.

Categorical data are presented as n (%) and comparisons were made using the chi-squared or Fisher's exact test. Numeric data are presented as the mean (SD) or median (interquartile range) depending on the distribution of the data and comparisons were made using the two sample independent t-test or the Wilcoxon rank-sum (Mann–Whitney) test. All test were two-tailed. $P < 0.05$ was considered statistically significant. Statistical analysis was performed using STATA, version 16 (StataCorp, College Station, TX, USA).

Results

In total, 100 patients were included in the present study, with 50 patients in each group. Data were collected for 7 days for each patient for a total of 700 days. The groups were matched for age, gender, BMI and illness severity. The reason for admission was similar in both groups: surgery or severe respiratory failure (Table 1).

Table 1 Baseline characteristics

Patient characteristics	1 % (n = 50)	2% (n = 50)	P value
Age (years), mean (SD)	52 (18.62)	56 (17.41)	0.30
Gender, male n (%)	31 (62%)	32 (64%)	0.84
Body mass index (kg m^{-2}), mean (SD)	27.4 (5.96)	27.6 (5.83)	0.89
Primary admission diagnosis, n (%)			
Surgery elective	18 (36%)	21 (42%)	0.54
Surgery unplanned	1 (2%)	5 (10%)	0.20
Severe respiratory failure	19 (38%)	16 (32%)	0.53
Infection	18 (36%)	11 (22%)	0.12
Surgery	1 (2%)	1 (2%)	
Asthma	0	2 (4%)	
Interstitial lung disease	0	2 (4%)	
Cardiology	12 (24%)	8 (16%)	0.32
Extracorporeal membrane oxygenation	15 (30%)	15 (30%)	1.00
Baseline APACHE, mean (SD)	27(7.03)	25 (6.18)	0.13
Number of comorbidities			
Cardiovascular	24	28	
Respiratory	13	18	
Neurological	5	6	
Endocrinology	15	18	
Renal	3	2	
Gastrointestinal	6	2	
Haematology	3	2	
Cancer	8	3	
Mental health disorder	8	5	
Other	13	11	

Propofol sedation

Table 2 contains the data on propofol dose and the associated energy and fat provision. The mean propofol dose delivered per day was similar in both. The dose was consistent across the first 5 days, irrespective of 1% or 2%.

All patients received propofol for 3 days in both groups; by day 5, four patients in each group were no longer receiving it. By day 7, 10 patients in the 1% group and 12 in the 2% group received no propofol infusions.

The patients in the 1% group received significantly more energy per day from propofol fat compared to the 2% group, contributing significantly towards to the total energy provision, providing 25% and 15% of total daily energy, respectively. The propofol dose per day over 7 days is shown in Fig. 1 and the impact that this has on energy received from propofol fat each day is shown in Fig. 2.

Nutrition therapy

The majority of nutrition therapy was provided as EN (97.3%) with the remaining as PN. Table 3 provides information on energy, protein and fat delivery. The mean energy and protein targets were not different between the two groups. The nutrition energy prescription was significantly less for the 1% compared to the 2% group, taking the additional energy from propofol fat into consideration to avoid overfeeding. The amount of protein delivered (g day^{-1}) was significantly higher in the 2% group, although it did not reach statistical significance for g kg^{-1} day^{-1} or percentage of target delivered (P = 0.09).

The delivery of nutrition volumes fell short in both groups, irrespective of propofol type, with the 1% group receiving 66% and the 2% group receiving 73% of target volume. Neither group achieved more than 80% of target protein, with the 1% group receiving 66.3% and the 2% group receiving slightly more at 71%. We calculated the amount of protein that the patients should have received if full prescribed volumes were delivered (accounting for propofol energy). The 2% group would have received significantly more with a mean of 82 g (1%) versus 97 g (2%) (P = 0.001).

All patients remained in the study for 7 days and should have received nutrition on each day. However, there were 40 days out of 700 days where no nutrition was delivered and there was no significant difference between the groups. The reason for non-delivery was a result of gastrointestinal intolerances and fasting of EN for procedures (e.g. bronchoscopies, transoesophageal echocardiogram, theatre and tracheostomies).

The amount of fat supplied was significantly greater in the 1% group, related to the volume of propofol solution provided as shown in Table 3. Ten percent of all patients exceeded the upper target of 1.5 g kg^{-1} day^{-1}, with the highest level of 2.1 g kg^{-1} seen in the 1% group. Thirty-six percent of patients receiving 1% propofol exceeded

Table 2 Propofol dose and energy provision

Propofol delivery	1 % (n = 50)	2% (n = 50)	P value
Propofol dose/per day (mg day^{-1}), mean (SD)	3003 mg (1399.73)	3224 mg (1383.72)	0.43
Energy from propofol (kcal day^{-1}), median (IQR)	353.9 (223.5–477.4)*	184.7 (118.2–243.9)*	<0.0001
Percentage of total energy target from propofol fat, mean (SD)	25.2 (10.88)	15.49 (8.38)	<0.0001

*Non-normally distributed data presented as median [interquartile range (IQR)]. and comparison using Wilcoxon rank-sum (Mann–Whitney) test.

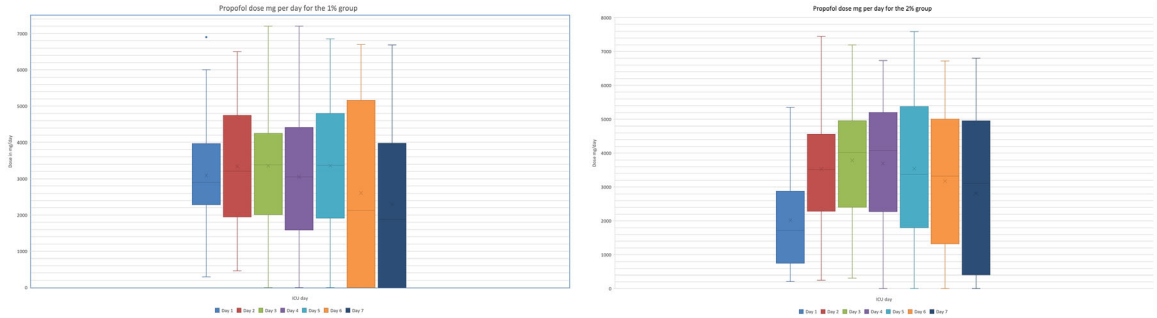

Figure 1 Comparison of propofol dose (mg day^{-1}) between the 1% and 2% groups. ICU, intensive care unit.

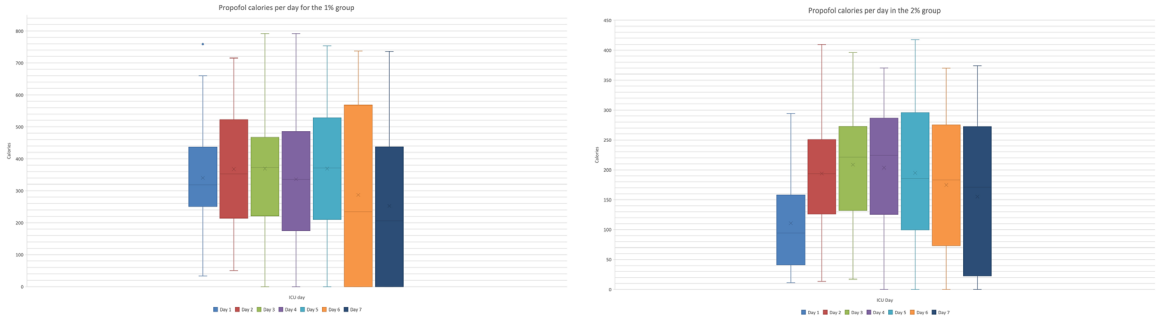

Figure 2 Comparison of propofol energy from fat (kcal day^{-1}) between the 1% and 2% groups. ICU, intensive care unit.

Table 3 Detail of nutrition therapy (energy, protein and fat) delivery by propofol group

	1 % (n = 50)	2% (n = 50)	P value
Energy targets and delivered			
Energy target (kcal kg^{-1}), mean (SD)	21.7 (6.8)	22.5 (5.1)	0.50
Energy delivered, (feed alone) (kcal kg^{-1}), mean (SD)	13.4 (5.6)	15.7 (5.1)	0.03
Energy delivered (feed + propofol) (kcal kg^{-1}), mean (SD)	15.5 (5.0)	17.5 (6.8)	0.09
Protein targets and delivered			
Protein target (g day^{-1}), mean (SD)	100.7 (31.66)	103.2 (23.55)	0.66
Protein target (g kg^{-1}), mean (SD)	1.3(0.45)	1.3 (0.32)	0.77
Protein delivered (g day^{-1}), mean (SD)	62.8 (24.68)	73.05 (25.16)	0.04
Protein delivered (g kg^{-1} day^{-1}), mean (SD)	0.8 (0.37)	0.9 (0.32)	0.09
Percentage of total energy target from protein, mean (SD)	15.3 (5.38)	16.89 (4.54)	0.11
Fat targets and delivery			
Total fat (feed + propofol) (g kg^{-1} day^{-1}), mean (SD)	0.9 (0.39)	0.72 (0.21)	0.034
Number of patients exceeding fat targets, n (%)			
1.5 g kg^{-1} day^{-1}	5 (10)	0	0.02
>45% total energy from fat	18 (36)	1 (2)	0.003
Percentage of total energy target from total fat (feed + propofol) Median (IQR)	42.7 (28.7–49.1)*	32.7 (27.1–37.7)*	0.001
Adequacy of nutrition delivery			
Percentage of nutrition volume mL delivered over 7 days, mean (SD)	66 (23.7)	73 (15.4)	0.10
Percentage energy target (feed alone) delivered over 7 days, mean (SD)	63.1 (22.28)	70 (16.87)	0.09
Percentage energy target (feed + propofol) delivered over 7 days, mean (SD)	82.8 (27.46)	71.50 (17.19)	0.016
Percentage protein target delivered over 7 days, mean (SD)	66.3 (26.83)	71.01 (25.47)	0.37
Number of patients who received >80% of protein targets over 7 days (%)	17 (34)	17 (34)	1

*Non-normally distributed data presented as median [interquartile range (IQR)] and comparison using Wilcoxon rank-sum (Mann–Whitney) test.

the predefined high level of 45% of total energy derived from fat compared to 2% of patients in the 2% propofol group. Propofol infusion syndrome was not detected in either group.

Discussion

The present study has demonstrated that switching from a 1% to 2% propofol solution decreased non-nutritional energy and increased protein delivery, which potentially become significant, if the other obstacles to feed delivery were removed. The dose of propofol was comparable for each of the two groups and, as predicted, there was a significant decrease in associated energy derived from fat for the 2% group. The nutrition energy prescribed was significantly less for the 1% compared to the 2% group, taking the additional energy from propofol fat into consideration to avoid overfeeding. Both groups had the same protein target and the amount of protein delivered (expressed as g day^{-1}) was significantly higher in the 2% group. Ten percent of all patients exceeded the upper fat target of 1.5 g kg^{-1} day^{-1} and 36% of patients receiving 1% propofol exceeded the predefined high level of 45% of total energy derived from fat. The delivery of nutrition target volumes fell short in both groups, resulting in patients receiving inadequate energy and protein irrespective of propofol dose.

Taylor *et al.* [7] investigated whether the use of 1% propofol rather than 2% reduced the numbers of patients achieving the protein dose in the mainly trauma cohort. Approximately 80% of patients received greater than 80% of energy and protein requirements. They also explored what the impact on protein delivery would be if they had used a 2% solution, suggesting that 98% of patients would receive 80% or more. Following a review of the literature and practice across the UK, our ICU switched from routinely using 1% propofol to the 2% formulation, aiming to optimise protein delivery. To our knowledge, we are the first to compare the nutritional provision in the same institution after switching from using 1% to 2% propofol. Our study adds to the existing evidence base by making the change to the propofol solution used in a clinical setting and by assessing nutrition delivery in a pragmatic manner as opposed to a theoretical model.

Our findings agree with those observed in other studies exploring the energy contribution of propofol infusions [5,7,11]. These studies highlight the large variation in the percentage of daily energy provided from the propofol energy, which is reflected by different critical care patient populations (e.g. medical compared to surgical or trauma). Our patient population had a higher illness severity compared to the other studies, which may explain the higher mean doses of propofol and the associated energy delivery observed. In the present study, patients in the 1% group received an average of 42% of total energy derived from fat with 36% of patients receiving in excess of this. This amount exceeds the 31% of the 1% cohort reported by Charriere *et al.* [5] but less than the 51% observed by Taylor *et al.* [7]. The lowest reported energy contribution was seen in a predominantly medical ICU patient cohort. The reported mean energy intake was 433 calories over the first 7 days, equating to an average of 62 kcal day^{-1} [11]. The largest retrospective study to compare propofol energy intake between two different ICUs, with one using a 1% solution and the other using a 2% solution, reported significant differences between the two units, with the 1% patients receiving 169 kcal day^{-1} and the 2% patients receiving 118 kcal day^{-1}.

We were able to significantly increase protein delivery (g day^{-1}) in our 2% patients. We did not achieve significance for the percentage of protein targets achieved as a result of the multiple obstacles experienced in nutritional delivery. The adequacy of nutritional delivery in our cohort was markedly lower than that achieved by Taylor *et al.* [7] and thus neither of our groups achieved 80% of the protein target. We do not consider that the lack of significant improvement in protein delivery should be reviewed as a failure; rather, it is a reflection of the challenges with respect to providing nutrition support to critically ill patients. Successful delivery requires strategies to overcome these obstacles. We demonstrated that, if the delivery had been optimal, the 2% group would have received significantly more protein at the same time as adjusting for propofol energy meeting greater than 80% of targets.

The volume of EN in our study and that of others [5,7,11] was reduced to account for the additional energy from propofol fat. Current evidence suggests that overfeeding is harmful, although the effects of a high-fat delivery on organ function are largely unknown. An excess of more than 45% of total energy derived from fat has been suggested as potentially harmful [5]. In the present study, 36% of patients received in excess of 45%. In a study comparing energy intake with patients receiving propofol and those who did not, the additional energy from propofol was not associated with higher mortality, duration of ventilation or ICU length of stay [6].

The major clinical concerns of overfeeding are increased CO_2 production from excessive carbohydrate (hence, prolonged ventilatory dependence) and poor glycaemic control. A recent systematic review found no evidence to suggest that overfeeding via the enteral route is associated with worse outcomes [12]. Very high fat, low carbohydrate diets (ketogenic diet) have historically been used to treat refractory status epilepticus, predominantly

in children [13]. More recently investigators are considering whether high-fat diets may have neurological benefits for some adults on intensive care units [14]. However, one of the observed side effects was metabolic acidosis [14], which is known to stimulate protein catabolism [15]. If the high-fat delivery from propofol is combined with an insufficient protein delivery, as frequently experienced in ICUs [16], this may aggravate the loss of muscle observed in critically ill patients.

Although our study investigated the impact that propofol energy may have on the overfeeding of total energy and under delivery of protein, in reality, it has highlighted suboptimal nutritional delivery. We observed an average of 69% of nutrition volume delivered, which was comparable to that seen in other propofol studies [5]. Internationally, it is a common phenomenon to fail to deliver nutrition targets to patients who are critically ill, with on average only 50–60% of energy and protein targets being received [16].

The reasons for this poor delivery and resulting energy and protein deficits are the frequent interruptions to enteral feeding for procedures and gastrointestinal intolerance [17,18]. When an energy and protein deficit accumulates, critically ill patients start experiencing ICU related complications [18] and poor recovery [19] Measures are needed such as ICU fasting guidelines to tackle unnecessary delays and interruptions to nutrition delivery [20,21].

Limitations

There are limitations to the present study. As a result of the retrospective observational design, some bias may have been introduced. We have tried to circumvent this by including patients who met the same inclusion criteria and were matched in terms of demographics and nutritional targets. We also had very low levels of drop out, supporting the validity and reliability. We only included those with an ICU stay longer than 3 days because we considered that the impacts of under and over feeding become more apparent with longer ICU stays. This limits the generisablity of our findings to those with shorter lengths of stay. Also, because our study participants were from a specialist cardiothoracic unit, which patients with high illness severity scores, it is not possible to make inferences about the generalisability of the results in general ICU patients.

We did not undertake a power calculation to determine the numbers needed to observe a true difference in treatments. Because the study design was retrospective, it was not designed to test the effects of the high-fat delivery on clinical outcomes such as length of mechanical ventilation and ICU stay. We did not collect data on triglyceride levels, which would have been very useful when comparing the effect of differing fat intakes. Finally, as a result of the complex nature of critically ill patients and the challenges of providing nutrition, we were unable to deliver the full nutrition targets that we set out to achieve.

Conclusions

Propofol sedation comes in a fat solution and can provide a significant fat and energy load. ICU nutrition guidelines advise adjusting nutritional plans to avoid excess propofol fat and the risk of harmful overfeeding. However, because EN products have fixed energy and protein ratios, reducing the EN energy delivered also reduces protein. It has been suggested that substitution of 2% for 1% propofol is a way to optimise protein intake. We conducted a retrospective pre- and post-observational study evaluating the impact on protein delivery when changing from a 1% to a 2% propofol solution. Both groups had the same protein target and the amount of protein delivered (g day^{-1}) was significantly higher in the 2% group. The 1% propofol group received significantly more fat energy, with 36% of patients exceeding high levels of 45% of total energy derived from fat. The delivery of nutrition target volumes fell short in both groups, resulting in patients receiving inadequate energy and protein irrespective of propofol dose.

Additional work is needed to consider the exact harm that high-fat delivery may pose, in light of the knowledge that nutrition delivery, particularly protein, is notoriously poor during critical illness. Without evidence of harm, it leads us to question the need to reduce volumes of EN, adjusting for propofol sources of energy. We recommend that an individual patient assessment is conducted assessing malnutrition status and adequacy of nutrition provision before restrictions are applied.

Conflict of interests, source of funding and authorship

The authors declare that they have no conflicts of interest.
No funding declared.
Both ET and CR formulated the study conception, and also participated in project administration, design, data acquisition, data analysis, writing the manuscript and agreeing on final approval of the version submitted for publication.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with STROBE. The lead author affirms that no important aspects of the study have been omitted and that any

discrepancies from the study as planned (approved by HRA and Health and Care Research Wales (HCRW) IRAS reference number: 286229, REC reference 20/HRA/3491) have been explained.

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GASTROINTESTINAL DISEASE

Habitual dietary fibre and prebiotic intake is inadequate in patients with inflammatory bowel disease: findings from a multicentre cross-sectional study

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Abstract

Background: Recommendations for dietary fibre intake in patients with inflammatory bowel disease are highly variable. Despite the potential benefits of prebiotic fibres on the gut microbiome, many patients with inflammatory bowel disease follow a low fibre diet. The present study comprehensively evaluated intakes of total and prebiotic fibres in patients with inflammatory bowel disease, aiming to determine the adequacy of fibre intake and factors that may influence intake.
Methods: Outpatients with a formal diagnosis of inflammatory bowel disease were recruited to this multicentre cross-sectional study. Habitual dietary fibre intake including prebiotic fibre types was measured using a validated comprehensive nutrition assessment questionnaire. Adequacy of total fibre intake was compared with Australian Nutrient Reference Values. Multiple linear regressions were performed to determine factors influencing fibre intake.
Results: Of 92 participants, 52% had Crohn's disease, 51% were male and the mean age was 40 years. Overall, only 38% of the cohort consumed adequate total fibre (median 24 g day^{-1}, interquartile range 18.5–32.9 g day^{-1}). Adequate fibre consumption was significantly less common in males than females (21.3% versus 55.6%, $P = 0.002$). Resistant starch intake (median 2.9 g day^{-1}, interquartile range 2.1–4.8 g day^{-1}) was significantly less than the proposed recommendations (20 g day^{-1}). Disease-related factors such as phenotype and disease activity were not found to influence fibre intake.
Conclusions: Patients with inflammatory bowel disease habitually consume inadequate fibre, particularly prebiotic fibre resistant starch. The potential deleterious effects of low prebiotic intake on the gut microbiome and disease-related outcomes in inflammatory bowel disease are unknown and warrant further research.

Introduction

Inflammatory bowel disease (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), comprises a chronic lifelong disease characterised by relapsing and remitting inflammation of the gastrointestinal tract [1–3]. The aetiology of IBD remains unclear, yet it is purported to involve a complex interaction between genetic, microbial, immunological and environmental factors [4,5]. Although the incidence and prevalence of IBD is highest

in developed countries, a rapid rise in incidence in developing countries has been observed over recent decades [4–6]. Such epidemiological trends implicate the key role of environmental factors in IBD susceptibility, particularly the widespread adoption of a Westernised diet [4,6].

Prospective cohort studies have shown that a low intake of fibre, as well as high intake of animal fats, processed foods and refined sugar, impart an increased risk of IBD [5]. Moreover, studies have shown that dietary factors, particularly fibre intake, are associated with a risk of clinical flare in patients with established IBD [7,8]. Habitual fibre intake shapes the constitution and function of the gut microbiome, providing substrate for short chain fatty acid (SCFA) production, which is vital to enterocyte function [9,10,11]. There is growing appreciation that the gut microbiome in patients with IBD differs from healthy controls, characterised by reduced diversity, labelled a dysbiosis [12].

Despite data supporting diet as a key factor in IBD susceptibility and disease course, aside from exclusive enteral nutrition, there is a scarcity of evidence for diet as therapy in IBD [13]. Consequently, patients with IBD are subject to highly variable and often contradictory dietary recommendations, which place them at risk of harmful dietary manipulation and food exclusions [14,15]. Accordingly, a recent study reported that 71% of patients with UC and 90% of patients with CD attempt some form of elimination diet [13]. Such restrictive diets place patients at risk of nutritional deficiencies and malnutrition [14,15]. International nutrition guidelines for IBD fail to provide recommendations for fibre intake; therefore, dietary fibre recommendations are proposed to be the same as those for the general healthy population [15,16].

Few studies have examined the habitual intake of fibre in patients with IBD. Existing studies, limited by sample size and heterogeneity, suggest that total fibre intake in people with IBD is less than healthy individuals and fibre recommendations [17]. Furthermore, beyond total fibre intake, few studies have comprehensively examined intakes of fibre subtypes [18–20]. Dietary fibre is defined as 'carbohydrates that are not hydrolysed or absorbed in the upper part of the gastrointestinal tract' [10,11]. Fibres can be classified as long chain or short chain, each exhibiting different effects in the gastrointestinal tract [11]. Long chain fibres include insoluble fibres (slowly or non-fermentable) and soluble fibres (moderately or highly fermentable), whereas short chain fibres include oligosaccharides (fructo- and galacto-), which are highly fermentable [11]. Fermentable fibres are termed prebiotics and defined as 'nutrients that favour the growth and predominance of beneficial microbes and their inherent functions, with the ability to induce health benefits to the host' [11,12]. The intake of these fibres in the IBD population is therefore of interest as a result of their potential to positively influence the composition of the gut microbiome [12]. Only one study has reported habitual fructo-oligosaccharide (FOS) intake in patients with CD, whereas no studies were identified to report on galacto-oligosaccharide (GOS) or resistant starch intake [21].

The overarching aim of the present study was to investigate the habitual fibre intake of patients with IBD. The key objectives of the study were to: (i) comprehensively evaluate habitual intakes of dietary fibre and fibre subtypes, including prebiotic fibres in patients with IBD; (ii) examine whether total fibre and resistant starch intake in patients with IBD is adequate in comparison with the available Australian recommendations; and (iii) identify factors that may be associated with the fibre intake of patients with IBD.

Materials and methods

Participants

Patients with a formal diagnosis of IBD managed across two tertiary IBD centres were recruited to this multicentre cross-sectional dietary study between March 2016 and October 2018. Patients were eligible for inclusion if they were aged 18 years and older, had a confirmed diagnosis of IBD and were able to complete a food frequency questionnaire (FFQ). Patients were excluded if they were pregnant, were breastfeeding or had a significant medical, psychiatric or cognitive co-morbidity that would hinder completion of questionnaires. Informed consent was obtained from all participants following eligibility screening. Patients were recruited from an existing observational study protocol, [22] as well as during IBD outpatient encounters and via distribution of newsletters.

Data collection

Basic demographic and clinical information was collected from participants using self-reported questionnaires, as well as via case note review, clinic letters and established IBD database records. Demographic data collected included age, gender, smoking status and whether participants had previously seen a dietitian for IBD-related advice. Clinical information included IBD phenotype, year of diagnosis, Montreal classification, [23] clinical disease activity score, previous bowel surgery, current medications and biomarkers of disease, activity including faecal calprotectin (FC) and C-reactive protein. Participants with CD were classified as having active disease if they had a Crohn's disease activity index score ≥ 150 or a Harvey–Bradshaw index score > 4 [24]. A simple clinical colitis activity index score > 2, or a Partial mayo score > 1, was used to classify active disease in participants with UC [24].

Dietary evaluation

Data on dietary intake were collected using the Comprehensive Nutrition Assessment Questionnaire (CNAQ), a self-administered 297-item semi-quantitative FFQ [25]. The CNAQ was developed by researchers at Monash University and was designed to assess dietary intake over a 12-month period [25]. The questionnaire was validated to accurately estimate more than 20 nutritional indices including fibre and prebiotic fibres [25]. Participants completed the CNAQ either electronically on the website, or on a paper-based version, which was then manually entered into the website by the researcher. Dietary intake data were exported into EXCEL, version 14.0.7214.5000 (Microsoft Corp., Redmond, WA, USA).

Intakes of soluble and insoluble fibre were manually calculated based on frequencies selected by participants, using food composition data [26,27]. A frequency fraction, based on the participants selection, was multiplied by the soluble and insoluble fibre content of each food contained in the CNAQ (for which food composition data were available). This provided total soluble and insoluble fibre intake data for all participants. Adequacy of total fibre intake was determined via comparison with fibre recommendations outlined in the Australian Nutrient Reference Values (NRV), which are set at an adequate intake (AI) [28]. Participants were classified as having an adequate fibre intake if they met the AI of 25 g day^{-1} for females or 30 g day^{-1} for males [28]. Total fibre intakes within the cohort were also compared with the general Australian population through 24-h recall data from the 2011–12 Australian Health Survey (AHS) [29]. In addition to total fibre, resistant starch intake was compared with proposed recommendations of 20 g day^{-1} as outlined in the Australian Resistant Starch Report by Landon *et al* [30]. Participants who reported dietary intakes deemed implausible were evaluated against energy intake cut-offs of <500 and >3500 calories day^{-1} and were excluded from analysis [31].

Statistical analysis

Categorical data were expressed as frequency and percentage and compared between disease phenotypes using the chi-square test of independence. Continuous data were tested for normality using histograms, skewness and kurtosis and reported as the mean (SD) or median and interquartile range (IQR) as appropriate. Continuous variables were compared between disease phenotypes using an independent samples *t*-test for parametric data and the Mann–Whitney *U*-test for non-parametric data.

Multivariable linear regression was performed to determine factors that may influence fibre intake in the IBD cohort. Variables included were age, gender, IBD phenotype, clinical disease activity, FC and whether participants had seen a dietitian for IBD advice. A second multivariable linear regression was conducted in participants with CD only, allowing the inclusion of two additional variables specifically relevant to this population group: disease behaviour and previous bowel surgery. The unstandardised coefficient, 95% confidence intervals (CI) and statistical significance were reported. The relationships between each variable and fibre intake were tested in a univariable model; however, all predictor variables were then entered into the multivariable regression, regardless of statistical significance in univariable models, as a result of their known clinical relevance. All assumptions of the multivariable linear regression model were checked for both regressions. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS, version 25 (IBM Corp., Armonk, NY, USA).

Although a formal power calculation was not performed, a sample size of 100 was employed based on an estimate of 10–15 participants per predictor variable to be examined in multiple regressions, providing adequate power for statistical analysis.

Ethical statement

This study was approved by the Central Adelaide Local Health Network Human Research Ethics Committee (HREC/16/RAH/24 2016) prior to commencement.

Results

Inflammatory bowel disease cohort

In total, 112 participants were recruited, 20 of whom were excluded on the basis of reporting implausible dietary intakes, leaving 92 participants for the analysis, comprising 48 (52.2%) with CD and 44 (47.8%) with UC (Table 1). The mean (SD) age of participants was 40 (13.7) years and 51.1% of participants were male. The median disease duration was 10 years and the majority of participants had never smoked (59.7%). There were no significant differences in demographic characteristics, including age, gender and smoking status between phenotypes. There were 16 participants (17.4%) who had undergone previous bowel surgery, all with CD. Most participants were classified as being in remission according to clinical indices (72.4%). Significantly more participants with CD (95.8%) were in remission compared to those with UC (43.6%) ($P \leq 0.001$). Accordingly, median FC levels were lower in participants with CD (87.0 μg g^{-1}) than with UC (195.0 μg g^{-1}) ($P = 0.015$). Only 33.7% had previously seen a dietitian for IBD advice, with no significant difference between phenotypes.

Dietary fibre intake

The median intake of total dietary fibre was 24.0 g day^{-1} (IQR 18.5–32.9 g day^{-1}) (Table 2). Insoluble and soluble fibre intakes were relatively proportionate with median intakes of 8.1 g day^{-1} (IQR 6.0–12.1 g day^{-1}) and 7.9 g day^{-1} (IQR 5.6–11.1 g day^{-1}), respectively. The median intake of resistant starch, 2.9 g day^{-1} (IQR 2.1–4.8 g day^{-1}), was shown to be lower than other fibre subtypes. There were no significant differences in fibre subtype intakes between phenotypes. The median intake of total oligosaccharides was 3.4 g day^{-1} (IQR 2.5–4.6 g day^{-1}), with FOS intakes of 2.3 g day^{-1} (IQR 1.7–3.2 g day^{-1}) and 1.0 g day^{-1} for GOS (IQR 0.7–1.4 g day^{-1}). There were no significant differences for intakes of oligosaccharides between phenotypes. Daily intakes of other FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) were also analysed and are provided in the Supporting information (Table S1).

Adequacy of dietary fibre intake

When compared with the Australian NRVs for total fibre intake, [28] 38% of participants were classified as having an adequate fibre intake (Fig. 1). There was a significant difference between the proportion of males (21.3%) and females (55.6%) who met the recommendations ($P = 0.002$). Median intakes of resistant starch within the

Table 1 Demographic and clinical characteristics of participants

	Total ($n = 92$)	CD ($n = 48$)	UC ($n = 44$)	*P* value
Males, *n* (%)	47 (51.1)	24 (50.0)	23 (52.3)	0.993†
Age, years, mean (SD)	39.9 (13.7)	39.3 (12.3)	40.8 (15.2)	0.613§
Smoking status, *n* (%)				0.359†
Never smoked	46 (59.7)	23 (53.5)	23 (67.7)	-
Current smoker	11 (14.3)	8 (18.6)	3 (8.8)	-
Ex-smoker	20 (26.0)	12 (27.9)	8 (23.5)	-
Disease duration, median (IQR)	10.0 (5.0–16.8)	12.0 (5.0–16.8)	9.0 (4.3–16.8)	0.298‡
Montreal classification				
Age of diagnosis, *n* (%)				
A1 (≤16 years)	–	9 (19.6)	6 (15.4)	-
A2 (17–40 years)	–	31 (67.4)	24 (61.5)	-
A3 (>40 years)	–	6 (13.0)	9 (23.1)	-
Montreal classification, *n* (%)		L1: 12 (26.1), B1: 22 (47.8) L2: 12 (28.3), B2: 10 (21.8) L3: 21 (45.6), B3: 14 (30.4)	E1: 5 (12.8) E2: 19 (48.7) E3: 15 (38.5)	-
L4 (upper gastrointestinal modifier)	–	3 (6.5)	–	–
P (perianal modifier)	–	10 (21.7)	–	–
Clinical disease activity, *n* (%)				<0.001†,*
Remission	63 (72.4)	46 (95.8)	17 (43.6)	-
Active disease	24 (27.6)	2 (4.2)	22 (56.4)	-
C-reactive protein, mg L^{-1}, median (IQR)	1.8 (0.4–6.6)	2.2 (0.4–7.3)	1.7 (0.5–6.5)	0.985‡
Faecal calprotectin, μg g^{-1}, median (IQR)	103.0 (22.3–391.0)	87.0 (20.0–172.3)	195.0 (46.0–720.3)	0.015‡,*
Current medication, *n* (%)				
Steroids	10 (11.2)	2 (4.3)	8 (18.6)	0.045¶,*
5-Aminosalicylates	45 (50.6)	15 (32.6)	30 (69.8)	0.001†,*
Immunomodulators	11 (12.4)	6 (13.0)	5 (11.6)	1.000†
Biologics	29 (32.6)	23 (50.0)	6 (14.0)	0.001†,*
Previous bowel surgery, *n* (%)	16 (17.4)	16 (33.3)	0 (0.0)	<0.001¶,*
Seen dietitian for IBD-related advice, *n* (%)	31 (33.7)	18 (37.5)	13 (29.5)	0.558†

Montreal Classification, a system to characterise the disease of patients with inflammatory bowel disease [23]; L1, ileal disease; L2, colonic disease; L3, ileocolonic disease; B1, non-stricturing/penetrating disease; B2, stricturing disease; B3, penetrating disease; E1, proctitis; E2, left-sided disease; E3, pancolitis. *P* value derived from comparing Crohn's disease and ulcerative colitis participants.

CD, Crohn's disease; IBD, inflammatory bowel disease; IQR, interquartile range; UC, ulcerative colitis.

†Chi-squared test of independence.

‡Mann–Whitney *U*-test.

§Independent samples *t*-test.

¶Fisher's exact test.

*Indicates statistical significance ($P < 0.05$).

Table 2 Intake of total fibre and fibre subtypes (g day^{-1}) amongst participants

Daily intakes, median (IQR)	Total (n = 92)	CD (n = 48)	UC (n = 44)	P value[†]
Total fibre	24.0 (18.5–32.9)	23.6 (19.2–33.2)	25.0 (18.4–32.9)	0.719
Insoluble fibre	8.1 (6.0–12.1)	9.8 (6.2–13.9)	7.6 (5.9–10.5)	0.133
Soluble fibre	7.9 (5.6–11.1)	9.7 (5.7–11.6)	7.1 (5.5–9.7)	0.227
Resistant starch	2.9 (2.1–4.8)	3.3 (2.2–4.9)	2.8 (2.0–4.0)	0.293
Total Oligosaccharides	3.4 (2.5–4.6)	3.5 (2.6–4.6)	3.3 (2.3–4.5)	0.600
FOS	2.3 (1.7–3.2)	2.5 (1.8–3.2)	2.2 (1.6–3.3)	0.827
GOS	1.0 (0.7–1.4)	1.0 (0.7–1.7)	1.0 (0.6–1.3)	0.488

P value derived from comparing Crohn's disease and ulcerative colitis participants.
CD, Crohn's disease; FOS, fructo-oligosaccharides; GOS, galacto-oligosaccharides; IQR, interquartile range; UC, ulcerative colitis.
[†]Mann–Whitney U-test.

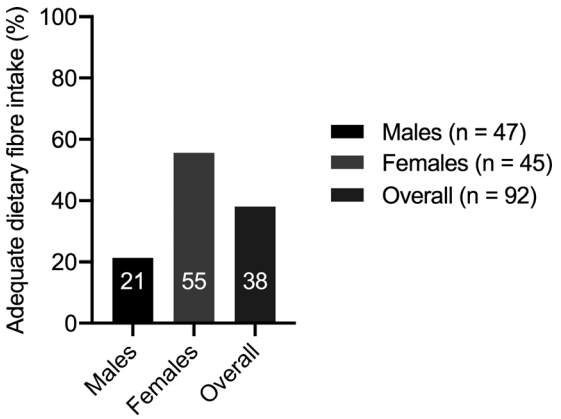

Figure 1 Adequacy of fibre intake within the inflammatory bowel disease cohort compared with Australian Nutrient Reference values for total dietary fibre intake [28].

examined cohort (2.9 g day^{-1}, IQR 2.1–4.8 g day^{-1}) were significantly lower than proposed recommendations of 20 g day^{-1} [30]. When total fibre intake was compared to the general Australian population (2011–12 AHS data), [29] female IBD participants were found to have numerically higher intakes (see Supporting information, Table S2). By contrast, male participants were found to have numerically lower intakes of total fibre in all age groups, except those aged 31–50 years old.

Factors influencing dietary fibre intake

There were no significant associations found between total fibre intake and predictor variables in either the univariable or multivariable models, as performed in the 61 participants for whom complete data for predictor variables was available (Table 3). Similarly, no significant associations were evident in the regression performed in only patients with CD (n = 48) in either the univariable or multivariable models, including those with and without previous bowel surgery (see Supporting information, Table S3).

Discussion

This cross-sectional study has demonstrated that the majority of outpatients with IBD do not consume adequate dietary fibre according to population-based guidelines [28]. Overall, only 38% of participants had adequate fibre intake and there was a significant gender imbalance, with significantly fewer men (21% versus 55%) meeting target intakes. Perhaps of more concern, low intake of prebiotic resistant starch was prevalent in the examined cohort. Given the likely deleterious impact of this on the gut microbiome, this possibly contributes to the well-recognised dysbiosis in IBD.

Few studies have explored habitual dietary fibre intake in patients with IBD, reporting a broad range of fibre intakes from 8.8 to 21.5 g day^{-1} [32,33]. The present study reported a median fibre intake of 24 g day^{-1}, which is below population-based recommendations, yet higher than habitual intakes reported in other studies. This may relate to properties of the CNAQ, which has been shown to over-estimate intakes of total fibre by up to 30% [25]. Comparisons across current studies are limited by small sample sizes, variable IBD populations, heterogeneity of dietary assessment tools and variable assessment time-periods. Nonetheless, the present study is the first to report habitual fibre intake in an Australian IBD population, providing valuable regional data with respect to informing the need for dietetic involvement in IBD care.

In the present study, we demonstrated that almost two-thirds of patients with IBD (62%) are falling short of recommended intakes for total fibre. Similarly, existing studies have found that <20% of participants met their national recommendations for fibre [17,34–36]. Despite failing to meet recommended intakes, insufficient fibre consumption amongst patients with IBD may be no greater than that observed in the general Australian population. A secondary analysis of the Australian 2011–12 National Nutrition and Physical Activity Survey, reported that 70% of adults did not meet recommendations for total fibre

Table 3 Multivariable linear regression investigating factors that influence fibre intake in an inflammatory bowel disease cohort

	Total fibre intake (g day^{-1})					
	Univariable model			Multivariable model		
Predictor variables	B	95% CI	*P* value	B	95% CI	*P* value
Age, years	0.175	−0.06 to 0.41	0.138	0.223	−0.03 to 0.48	0.084
Gender, *Male*						
Female	1.375	−3.94 to 6.69	0.606	1.459	−3.95 to 6.87	0.591
Phenotype, *ulcerative colitis*						
Crohn's disease	0.144	−5.27 to 5.56	0.958	−2.482	−10.09 to 5.13	0.516
Disease activity, *remission*						
Active disease	−1.376	−7.30 to 4.55	0.644	−4.857	−13.53 to 3.81	0.266
Faecal calprotectin, μg g^{-1}	0.0004	−0.004 to 0.004	0.837	0.001	−0.003 to 0.006	0.636
Seen a dietitian before, *no*						
Yes	0.587	−4.86 to 6.03	0.830	1.491	−4.14 to 7.12	0.598

Regression performed in 61 participants for whom complete data for predictor variables was available. Italicised groups represent reference categories in regression.
B, unstandardised coefficient; CI, confidence interval.

[37]. Furthermore, females were 2.2-fold more likely to meet fibre recommendations than males, which was consistent with our findings [37]. The observed gender difference may be explained by lower food literacy in males, with lower levels of nutrition knowledge and health consciousness [37].

The present study provides a comprehensive account of habitual fibre subtype intakes in patients with IBD. Current fibre recommendations outlined in the NRVs do not provide specific targets for resistant starch intake; however, the Australian Resistant Starch report proposes optimal bowel health may require intakes of 20 g day^{-1} [30]. This is considerably higher than the median intake of 2.9 g day^{-1} in the examined IBD cohort, demonstrating a limited intake of prebiotic fibre. Two previous studies by Barrett *et al.* [38] and Anderson *et al.* [21] have classified low FOS intakes as ≤1.3 and ≤2.69 g day^{-1}, respectively, which are comparable with intakes in the examined IBD cohort (median 2.3 g day^{-1}). Additionally, Barrett *et al.* [38] established low GOS intakes as ≤0.3 g day^{-1}, which is lower than the median intake of 1.0 g day^{-1} in the examined IBD cohort [38]. Notably, it is difficult to determine whether the intake of prebiotic oligosaccharides in the examined cohort is insufficient, with a lack of prior studies, recommendations or consensus guidelines against which we can compare our findings.

Although the present study was not able to demonstrate specific factors influencing fibre intake in patients with IBD, several underlying reasons may be proposed. First, many patients with IBD are broadly advised by clinicians to avoid or reduce their fibre intake [14,15,16]. This advice is based on historical recommendations that a 'low residue' diet is useful for short-term control of gastrointestinal symptoms and to reduce stool output [39]. However, in many instances, patients are not advised to re-introduce fibre, which can result in prolonged unnecessary fibre restriction [39]. Such advice is compounded by the Internet, which is fraught with dietary recommendations that are not supported by evidence. A review by Hou *et al.* [40] identified 32 websites discussing recommendations for fibre intake, with 72% of these recommending avoidance of high fibre diets or foods. Second, more than 40% of patients with IBD suffer from concurrent irritable bowel syndrome (IBS), with concordantly high rates of dietary intolerances in the IBD cohort [41]. Avoidance of FODMAPs may be habitually undertaken by patients with IBD to mitigate functional gastrointestinal symptoms [21,38]. This is often without dietetic support, leading to unnecessary dietary restriction and reduced intakes of fibre, particularly prebiotic short chain fibres (oligosaccharides). Third, some patients with IBD have stricturing disease, for whom a low fibre diet is recommended to reduce the risk of intestinal obstruction [16].

An inadequate consumption of prebiotic fibres deprives the gut microbiome of key fermentation substrates for the production of SCFAs [42,43]. SCFAs reduce the pH within the intestinal lumen, promote the growth of health-promoting species, and are imperative for intestinal epithelial cell turnover, cell differentiation and maintaining the mucosal barrier through tight junctions [11,44–46]. Butyrate is the primary energy source of enterocytes, with evidence of anti-inflammatory and anti-tumour effects within the colon, which is relevant for people with IBD who suffer chronic inflammation and an increased risk of malignancy [12,44]. Patients with IBD have been demonstrated to have a relative paucity of butyrate-producing bacteria such as *Faecalibacterium pruasnitzii* and

those in clostridial clusters IV and XIVa [47]. Therefore, low intakes of prebiotic fibres may potentiate this so-called bacterial dysbiosis in patients with IBD.

In the absence of strong clinical indication, patients with IBD should be discouraged from following historical low fibre diets. A gastrointestinal specialist dietitian is well placed to support patients to overcome food anxiety and liberalise dietary intake when a restrictive diet is being followed [15]. Further research is required to explore whether manipulation of prebiotic content within the diet can lead to favourable changes in the gut-microbial composition and function in IBD, and whether this yields real clinical benefit. To assist with this, additional research and available food composition data for prebiotic fibres would be valuable because the scarcity of current data likely underestimates true intakes of fibre subtypes during analysis.

The use of the CNAQ, a comprehensive FFQ containing 297 items, allowed capture of a complete picture of dietary intake. Additionally, the CNAQ enabled quantification of prebiotic fibre intakes such as resistant starch, FOS and GOS, which have not been comprehensively explored in an IBD cohort [21]. Furthermore, the CNAQ measured dietary intake over a 12-month period, which is more reflective of usual dietary intake than shorter periods (1 week or 1 month) [48]. In terms of limitations, sample size reduction following application of cut-off values reduced the power for multivariable analyses and may have increased the risk of type II error. Recruitment of participants from pre-existing studies may have introduced sampling bias. Variable remission states between phenotypes may have influenced the study findings, with significantly more participants with CD in clinical remission. Measurement of disease activity at a single timepoint may not be reflective of longer-term disease activity over the 12-month period of dietary analysis. This reduces the ability to draw conclusions regarding relationships between disease activity and fibre intake. Furthermore, disease duration and concomitant IBS were not analysed as factors potentially contributing to fibre intake, nor were comparisons drawn between age and gender in terms of intake of fibre subtypes or resistant starch. Lastly, the use of FFQs to measure dietary intake is subject to recall and social desirability bias. FFQs also overestimate dietary intakes and therefore comparison with 24-h recall data from the AHS is a further limitation [25,48].

In conclusion, most patients with IBD, particularly males, habitually consume inadequate amounts of dietary fibre. Restricted intake of prebiotic fibres may have deleterious effects on the gut microbiome and contribute to dysbiosis in IBD. Widespread misguided advice for a low fibre diet in patients with IBD needs to be addressed and the findings of the present study add credence to the necessity for involvement of specialised dietitians in IBD care. Further studies are warranted to investigate the long-term effects of inadequate fibre and prebiotic intake in the IBD cohort, including effects on overall nutrient intake, the gut microbiome and IBD-related outcomes.

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Conflict of interests, source of funding and authorship

RD, ASD and AV declare that they have no conflicts of interest. JB runs a private practice, Diet Solutions, which provides dietary advice to patients with inflammatory bowel disease and other conditions. JB developed the CNAQ during PhD studies, and now holds an affiliate position with Monash University, Department of Gastroenterology, where the CNAQ is available for purchase by external researchers. SPC has received advisory, speaking fees or research support from Ferring, Microbiotica, Janssen. Shareholding: BiomeBank. JMA has received speaker's fees, research support, been on advisory boards for Abbott, AbbVie, Allergan, Anatara, AstraZeneca, Bayer, Celgene, Ferring, Gilead, Hospira, Immuninc, ImmusanT, Janssen, MSD, Nestle, Progenity, Pfizer, Sandoz, Shire, Takeda, Vifor, RAH Research Fund, The Hospital Research Fund. RVB has received Grant/Research support/Speaker fees (all paid to employer for research support): AbbVie, Ferring, Janssen, Shire, Takeda, Emerge Health. Shareholding: BiomeBank.
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RD, AD and RVB were involved in conception and design of the project. RD, AD, JB, AV, JA and RVB were involved in data acquisition, management analysis and interpretation. All authors were involved in drafting and revision of the article. All authors approved the final version of the manuscript submitted for publication.

Transparency statement

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with STROBE2 guidelines. The lead author affirms that no

important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Dietary intakes of FODMAPs (g day^{-1}) amongst participants

Table S2. Comparison of fibre intake (g day^{-1}) within the IBD cohort with the 2011–12 Australian Health Survey [29]

Table S3. Multivariable linear regression investigating factors that influence fibre intake in Crohn's disease participants

Video S1. Virtual Abstract

GASTROINTESTINAL DISEASE

The effect of immunonutrition in patients with acute pancreatitis: a systematic review and meta-analysis

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Keywords
acute pancreatitis, enteral nutrition, meta-analysis, parenteral nutrition, randomized controlled trials.

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Abstract

Background: The effect of immunonutrition is controversial compared to standard supplementation with respect to the management of patients with acute pancreatitis.
Methods: An online literature search on four databases (PubMed, Cochrane, Embase and Web of Science) was performed to identify all of the randomised controlled trials assessing the effects of enteral or parenteral immunonutrition in acute pancreatitis. A fixed or random effects model was chosen using REVMAN, version 5.3 (https://revman.cochrane.org). The count data were analysed using the risk ratio (RR) and 95% confidence interval (CI).
Results: Five hundred and sixty-eight patients were included via our search in which 14 articles matched our criteria for enrolling the meta-analysis. Immunonutrition significantly reduced the risk of organ failure (RR = 0.42; 95% CI = 0.26–0.70, $P = 0.0008$), infectious complications (RR = 0.78; 95% CI = 0.62-0.99; $P = 0.04$) and mortality (RR = 0.37; 95% CI = 0.21–0.66; $P = 0.006$). Length of hospital stay was also shorter in patients who received immunonutrition (mean difference = −1.73 days; 95% CI = −2.36 to −1.10; $P < 0.00001$). Total interventions of patients were decreased (RR = 0.73; 95% CI = 0.55–0.97; $P = 0.03$). Body mass index in patients with immunonutrition was reduced more than standard nutrition (mean difference = −2.00; 95% CI = −3.96 to −0.04; $P = 0.05$).
Conclusions: Immunonutrition support such as glutamine and ω-3 fatty acids is potentially beneficial with respect to improving clinical outcomes in patients with acute pancreatitis.

Introduction

Acute pancreatitis (AP) is a systemic immune inflammatory response to the autologous digestion of the pancreas and peri-pancreatic organs. It has become one of the most common diseases of the gastrointestinal tract, with significant emotional, physical and financial consequences to patients [(1)]. A pro-inflammatory response characterised by a release of large amounts of cytokines occurs at the early stage of severe AP, triggering systemic inflammatory response syndrome and subsequent organ failure [(2)]. Organ failure is associated with a mortality of up to 35% in AP [(3)].

As a result of the lack of an effective etiological intervention, supportive therapies remain the principal treatment strategy for these patients [(1,4,5)]. Experts have promoted nutritional support strategies in acute pancreatitis to an increasingly important position in the past years. Traditional nutritional supplementation essentially includes three approaches: enteral nutrition (EN), parenteral nutrition (PN) and no supplemental nutrition [(6)]. After more than half a century of development, clinical nutrition has evolved from an early focus on supplementation of energy and basic nutrients to the present exploration of personalised nutritional solutions that aim to improve the immune function and promote the recovery

of cell, tissue and organ functions, which has resulted in immunity nutrition receiving more and more attention from clinicians [7,8,9,10]. Examples of special nutritional substrates capable of modifying immune function include glutamine, arginine, ω-3 polyunsaturated fatty acids (ω-3 PUFA) and nucleotides collectively, through their own pharmacological effects with respect to stimulating immunity cells, enhancing the function of immune response, maintaining a normal and moderate immune response, regulating body metabolism, reducing harmful or excessive inflammatory reactions, protecting the integrity of the intestinal barrier function, and reducing the role of bacterial translocation, together comprising 'immunonutrition' (ImN) [11].

Although enteral ImN (EIN) or parenteral ImN (PIN) recommendations for acute pancreatitis are positioned at the top of the findings of previous randomised controlled trials (RCTs) and meta-analyses [12,13,14], the latest 2018 European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines do not support routine immune nutrient supplementation for critical ill patients [15]. All of the evidence is based on previous studies mainly focused on the efficacy of single route InM supplementation or a single nutrient such as glutamine. Thus, there is still insufficient evidence to systematically assess whether ImN supplementation is beneficial or not, and many details remain to be clarified.

There is a need to fill the gap regarding the existing evidence of EIN and PIN in the setting of acute pancreatitis, update the data on the relative value of EIN and PIN, and explore the main clinical effects of ImN. In the present study, the effect of ImN on the key clinical outcomes was systematically reviewed via three random comparisons (PIN versus PN, EIN versus EN, EIN versus PIN).

Materials and methods

The present study was performed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol for reporting systematic reviews and meta-analyses [16]. The review was registered in the PROSPERO database (CRD42020197320).

Search strategy and selection

To identify relevant publications earlier than June 2019, two independent investigators performed a systematic literature search in the following online databases: EMBASE, PubMed, Cochrane Library and Web of Science. The following terms were used in the search: ("immunonutrition" OR "immune-enhancing diet" OR "immune nutrients" OR "nutritional support" OR "dietary supplementation" OR "fatty acids, omega-3 [Mesh]" OR "fish oil" OR "L-glutamine" OR "glutamine" OR "nucleotides" OR "RNA") AND ("acute pancreatitis"). No restriction in terms of time of publication was considered. No language restriction was applied and the search was limited to human studies. In addition, the reference lists of related articles were screened to avoid missing any publication. The eligibility criteria for articles to be selected were parallel-group RCTs in which an EIN or PIN solution was compared with the standard form in patients with acute pancreatitis.

Inclusion and exclusion criteria

Among the articles with the subject of ImN in acute pancreatitis, we selected those consistent with the inclusion and exclusion criteria:

Inclusion criteria:

-RCTs which used EIN or PIN containing glutamine or glutamine dipeptide or arginine or nucleotides or RNA and compared its effects with standard EN or PN on clinical outcomes of patients with acute pancreatitis

-RCTs which used EIN or PIN containing u-3 FAs or fish oil and compared its effects with standard EN or PN on clinical outcomes of patients with acute pancreatitis

-Both ImN solution and standard form had to be isocaloric and also iso-nitrogenus

-Patients involved were females or males aged 16 years or over, with acute pancreatitis whom needed nutrition therapy, and the enteral or parenteral feeding had begun within 72 h

-RCTs that had our desirable clinical outcomes [organ failure, mortality rate, or length of hospital stay (LOS)]

Exclusion criteria:

-RCTs evaluated EN or PN, or compared EN with PN

-RCTs evaluated EIN or PIN in any other condition except acute pancreatitis or gathered all critically illnesses together

Quality assessment

REVMAN, version 5.3 (https://revman.cochrane.org) was used to evaluate the risk of bias, which contained seven items: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other biases. The risk of bias assessment

was carried out by two reviewers independently (Y.X and J.Z). A third reviewer (X.KL) arbitrated unresolved disagreements. The potential bias was graded as 'high risk', 'low risk' or 'unclear risk'.

Data extraction

Data extraction was conducted independently by two investigators using a standardised data collection form [17]. The baseline characteristic data such as the first author, study setting, year of publication, age, gender, number of patients in the intervention (ImN) and control (no-ImN) group, severity of AP, route of ImN administration, composition of nutrition formulation, duration of intervention, amount of dosage, and relevant outcomes were collected. Each patient population was used only once if it appeared in more than one publication.

Statistical analysis

REVMAN, version 5.3 software and STATA, version 12.0 (StataCorp, College Station, TX, USA) were used to analyse the collected data. Risk ratio (RR) and 95% confidence interval (CI) were used for dichotomous data. Continuous data were analysed by the weighted mean difference (MD) and 95% CI. Begg's test and Egger's test were used to finish the publication bias analysis. Heterogeneity among included studies was assessed by the Cochrane Q test and I^2 test. If $I^2 < 50\%$ or $P > 0.10$, heterogeneity was considered small and the fixed effects model was used. If $I^2 > 50\%$ or $P < 0.10$, heterogeneity was considered great and a random effects model was adopted. Subgroup analysis was performed to analyse sources of large heterogeneity and the different effects of EIN and PIN. For some studies [18,19] that only showed medians and ranges, we used the method provided by Hozo *et al.* [20] to estimate the mean (SD). $P < 0.05$ (two-tailed) was considered statistically significant.

Results

Study identification and selection

Six thousand one hundred thirty-one articles were extracted from PubMed, Embase, Web of Science and the Cochrane library. After removing the duplicates and screening the title and abstract, 35 articles were eligible for further assessment, of which 21 articles did not meet the inclusion criteria. Finally, 14 articles [18,19,21,22,23,24,25,26,27,28,29,30,31,32] were included in this meta-analysis. Figure 1 showet he process of literature selection.

Study characteristics

Table 1 shows essential characteristics of the 14 mentioned RCTs consisting of 568 participants enrolled in the meta-analysis, of whom 281 patients were randomised into ImN supplementation and 287 into patients to the no-ImN group (control group). Figure 2 summarises the risk of bias assessment in the included studies, most of which were of moderate quality.

Clinical outcomes

Impact on mortality

In total, 14 RCTs involving 568 patients mentioned the mortality of the ImN group and control group. There was no heterogeneity among these studies ($P = 0.96$, $I^2 = 0.0\%$). The meta-analysis of RCTs showed that ImN was markedly associated with mortality reduction in patients with acute pancreatitis (RR = 0.37; 95% CI = 0.21–0.66; $P = 0.0006$) (Fig. 3). Subgroup analysis showed EIN did not reduce mortality in patients with acute pancreatitis (RR = 0.49; 95% CI = 0.22–1.10; $P = 0.08$) (Fig. 3). PIN can reduce the risk of mortality significantly (RR = 0.29; 95% CI = 0.13–0.65; $P = 0.003$) (Fig. 3). Funnel plots did not reveal any evidence of publication bias (Begg's test, $P = 0.115$; Egger's test, $P = 0.015$).

Impact on infectious complications

Twelve studies including 495 patients compared infectious complications between the ImN group and control group. No heterogeneity was found among these studies ($P = 0.88$, $I^2 = 0.0\%$). The meta-analysis of RCTs showed that there was a significant difference on the infectious complications of two groups (RR = 0.78; 95% CI = 0.62–0.99; $P = 0.04$) (Fig. 4). Subgroup analysis was conducted to show the effect of EIN and PIN separately. The results obtained showed that EIN did not reduce infectious complications in patients with acute pancreatitis (RR = 0.92; 95% CI = 0.67–1.25; $P = 0.58$) (Fig. 4). PIN can reduce the risk of infectious complications significantly (RR = 0.65; 95% CI = 0.45–0.94; $P = 0.02$) (Fig. 4). Funnel plots did not show evidence of publication bias (Begg's test, $P = 0.945$; Egger's test, $P = 0.320$).

Impact on organ failure

Nine RCTs involving 402 patients mentioned the organ failure. There was no heterogeneity among these studies ($P = 0.70$, $I^2 = 0.0\%$). The meta-analysis of RCTs showed that ImN markedly reduced the mortality of patients with acute pancreatitis (RR = 0.42; 95% CI = 0.26–0.70; $P = 0.0008$) (Fig. 5). Subgroup analysis showed that both EIN and PIN reduced the risk of organ failure

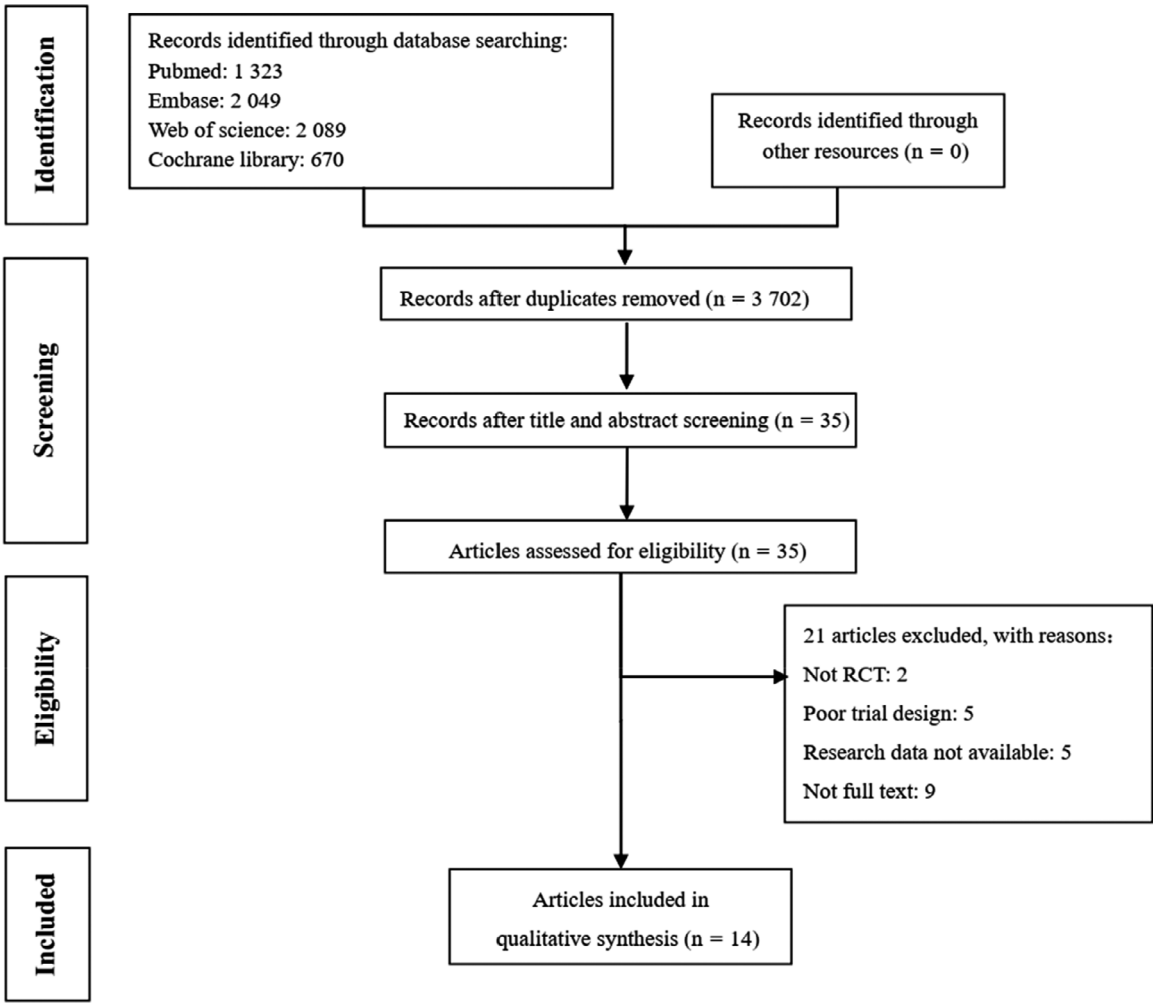

Figure 1 PRISMA flow diagram of study identification, inclusion and exclusion. RCT, randomised controlled trial.

significantly (EIN: RR = 0.40; 95% CI = 0.19–0.84; $P = 0.02$; PIN: RR = 0.45; 95% CI = 0.23–0.88; $P = 0.02$). Funnel plots did not reveal any evidence of publication bias (Begg's test, $P = 0.386$; Egger's test, $P = 0.127$).

Impact on length of hospital stay

Eleven studies including 454 patients compared the LOS in hospital between the ImN group and control group. There was no heterogeneity across studies ($P = 0.06$, $I^2 = 43.0\%$). The meta-analysis of RCTs showed a significant association between ImN and short LOS in hospital (MD = −1.73 days; 95% CI = −2.36 to −1.10; $P < 0.00001$) (see Supporting information, Fig. S1). Subgroup analysis was conducted to show the effect of EIN and PIN separately. The results obtained showed that EIN did not reduce the LOS in hospital (MD = 0.31 days; 95% CI = −2.24 to 2.86; $P = 0.81$) (see Supporting information, Fig. S1). PIN could reduce the LOS in hospital in patients with acute pancreatitis (Fig. S1; MD = −1.87 days; 95% CI = −2.51 to −1.22; $P < 0.00001$) (see Supporting information, Fig. S1). Funnel plots did not reveal any evidence of publication bias (Begg's test, $P = 0.640$; Egger's test, $P = 0.855$).

Impact on total interventions

Four RCTs with 80 patients mentioned the interventions. No heterogeneity was found across these studies ($P = 1.00$, $I^2 = 0.0\%$). The meta-analysis showed that ImN markedly reduced interventions of patients with acute pancreatitis (RR = 0.73; 95% CI = 0.55–0.97; $P = 0.03$) (see Supporting information, Fig. S2).

Impact on body mass index

Two RCTs enrolled in meta-analysis mentioned the body mass index (BMI). There was no heterogeneity between the studies ($P = 1.00$, $I^2 = 0\%$). The meta-analysis showed that ImN reduced BMI in patients with acute

Table 1 Characteristics of RCTs included in meta-analysis.

Study	Country	Year	Total number of patients	Patients (IN/NN)	Male/ female	Severity criteria used	Route of nutrition	Intervention	Dosage	AD–IN interval	Duration of intervention	Outcome
Arutla *et al.* (32)	India	2019	40	18/22	37/3	APACHE II, Glasgow, SOFA	Enteral	Gln composite	0.6 g kg^{-1} day^{-1}	<48 h	7 days	d, e, f, g, h, i
Singh *et al.* (18)	India	2014	80	41/39	49/31	APACHE II, CTSI	Enteral	Gln alone	NR	<48 h	7 days	c, d, f, g, h
Hajdú *et al.* (31)	Hungary	2012	45	24/21	42/3	Glasgow	Enteral	Gln alone	0.5 g kg^{-1} day^{-1}	48–72 h	7 days	d, f, h, i
Wang *et al.* (30)	China	2009	56	28/28	39/17	APACHE II	Parenteral	Omega-3 fatty acids	0.2 g kg^{-1} day^{-1}	<72 h	5 days	d, f, h, i
Wang *et al.* (29)	China	2008	40	20/20	28/12	APACHE II	Parenteral	Omega-3 fatty acids	0.2 g kg^{-1} day^{-1}	<72 h	5 days	d, f, g, h
Fuentes-Orozco *et al.* (26)	Mexico	2008	44	22/22	24/20	APACHE II, Ranson, CTSI	Parenteral	Gln alone	0.4 g kg^{-1} day^{-1}	24-48 h	10 days	a, c, d, e, f, g, i
Huang *et al.* (27)	China	2008	32	14/18	17/15	APACHE II	Enteral	Gln + Arg	0.3 g kg^{-1} day^{-1}	<72 h	At least 2 weeks	c, d, f, g, h
Yang *et al.* (28)	China	2008	50	25/25	31/19	APACHE II, Ranson, CTSI	Parenteral	Gln alone	0.5 g kg^{-1} day^{-1}	<48 h	7 days	a, f, g
Sahin *et al.* (25)	Turkey	2007	40	20/20	17/23	Ranson	Parenteral	Gln alone	0.3 g kg^{-1} day^{-1}	NR	At least 1 week	a, b, c, d, f, g, h
Pearce *et al.* (24)	UK	2006	31	15/16	18/13	APACHE II	Enteral	Gln composite	NR	<72 h	Clinically indicated	a, e, f, g
Lasztity *et al.* (23)	Hungary	2005	28	14/14	16/12	APACHE II, CTSI	Enteral	*n*-3 PUFAs	NR	<48 h	5–7 days	c, d, f, g, h
He *et al.* (22)	China	2004	41	20/21	22/19	NR	Parenteral	Gln alone	0.4 g kg^{-1} day^{-1}	24-48 h	At least 2 weeks	a, d, f, g, h
Ockenga *et al.* (19)	Germany	2002	28	14/14	16/12	APACHE II, CTSI	Parenteral	Gln alone	0.3 g kg^{-1} day^{-1}	<72 h	At least 1 week	a, b, c, d, f, g
De Beaux *et al.* (21)	UK	1998	13	6/7	8/7	Glasgow	Parenteral	Gln alone	0.2 g kg^{-1} day^{-1}	NR	1 week	d, f

a, albumin; b, body mass index (BMI); c, C-reactive protein; d, infectious complication; e, interleukin (IL)-6; f, mortality; g, length of hospital stay; h, organ failure; i, total intervention. IN, immunonutrition group; NN, no immunonutrition group; NR, not reported; APACHE, Acute Physiology And Chronic Health Evaluation; CTSI, Computed Tomography Severity Index; AD–IN interval, the interval between admission and start of immunonutrition; Gln, glutamine; Arg, arginine.

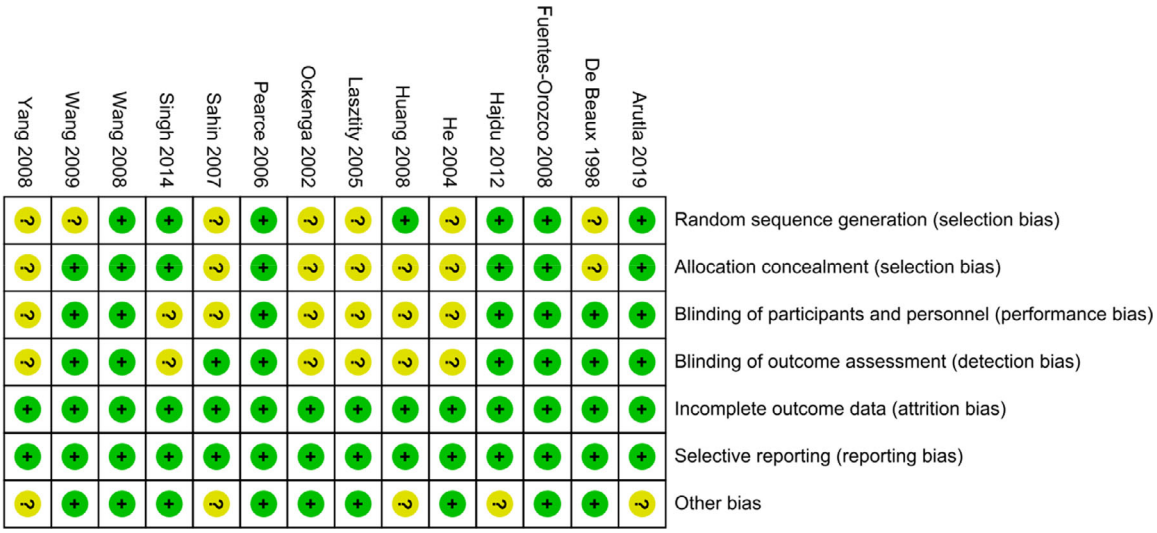

Figure 2 Methodological quality of the randomised controlled trials included in the systematic review.

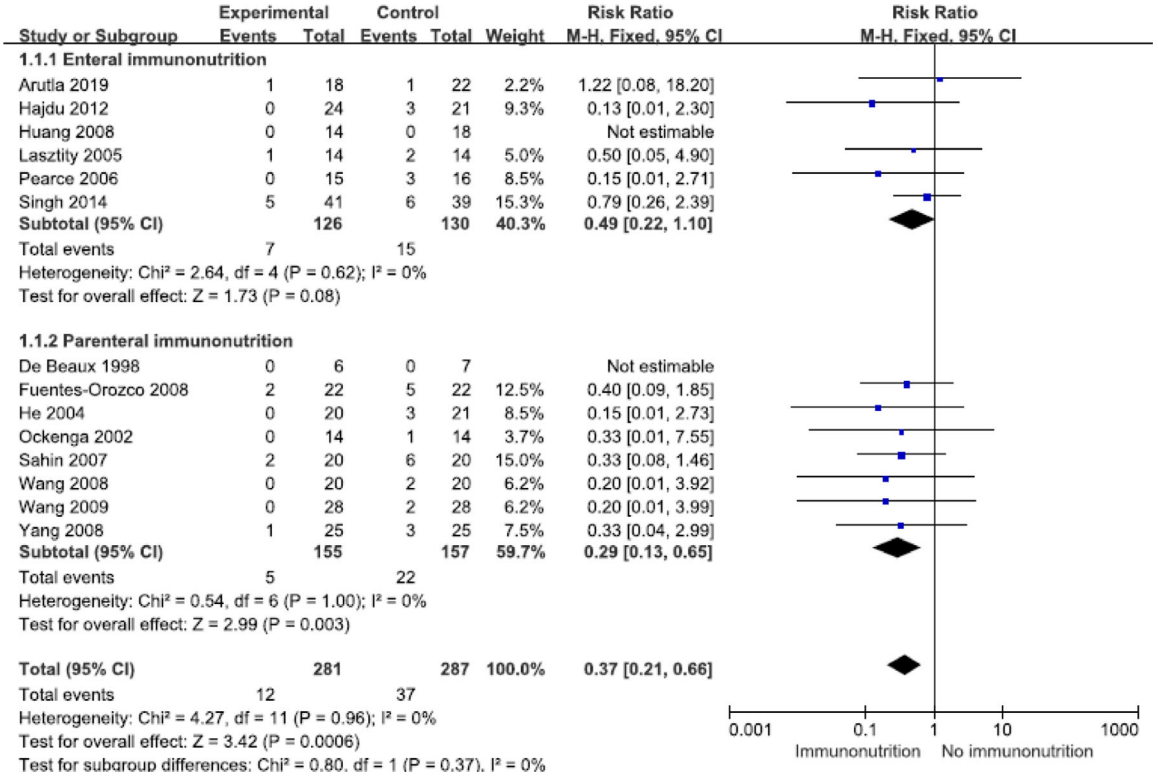

Figure 3 Forest plot of effect of immunonutrition on mortality.

pancreatitis (MD = −2.00; 95% CI = −3.96 to −0.04; $P = 0.05$) (see Supporting information, Fig. S3).

Impact on laboratory index

The three laboratory indices [albumin, interleukin (IL)-6, C-reactive protein (CRP)] assessed in the 14 articles are shown in the Supporting information (Table S1). Six RCTs with 234 patients mentioned the albumin. The data were considered to be heterogeneous ($P < 0.00001$, $I^2 = 92.0\%$) and so a random-effect model was adopted. The meta-analysis showed that no significant differences between two groups in term of albumin

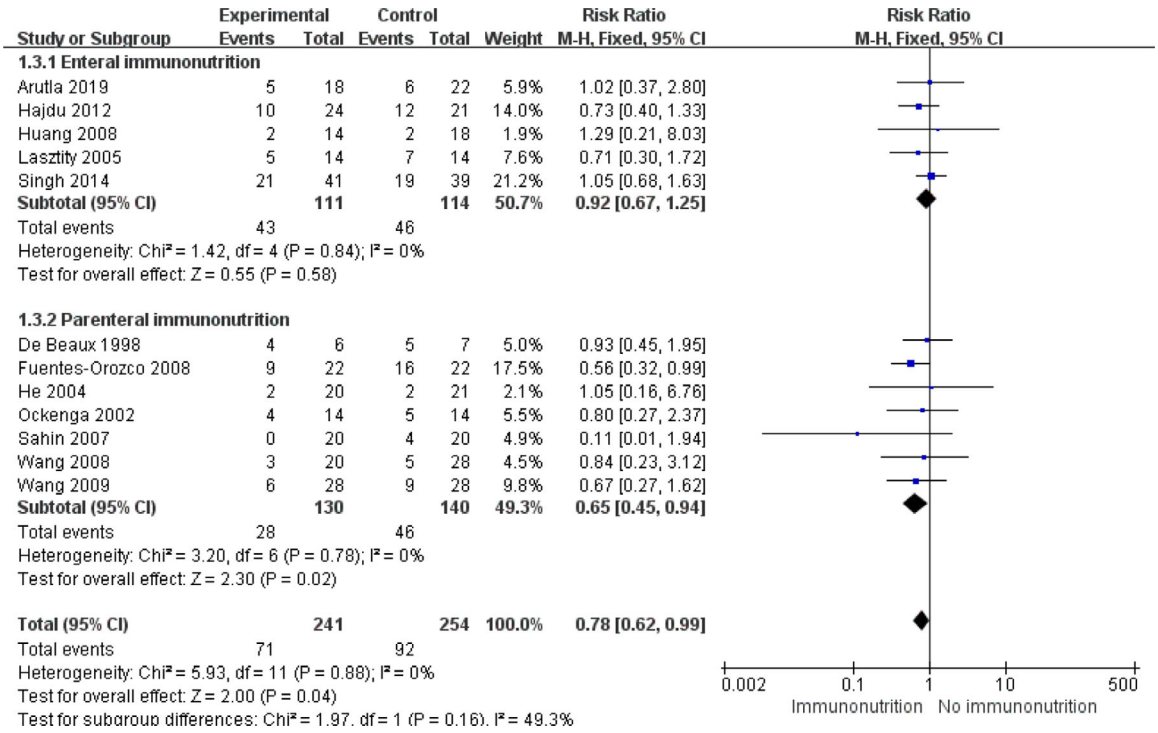

Figure 4 Forest plot of effect of immunonutrition on infectious complications.

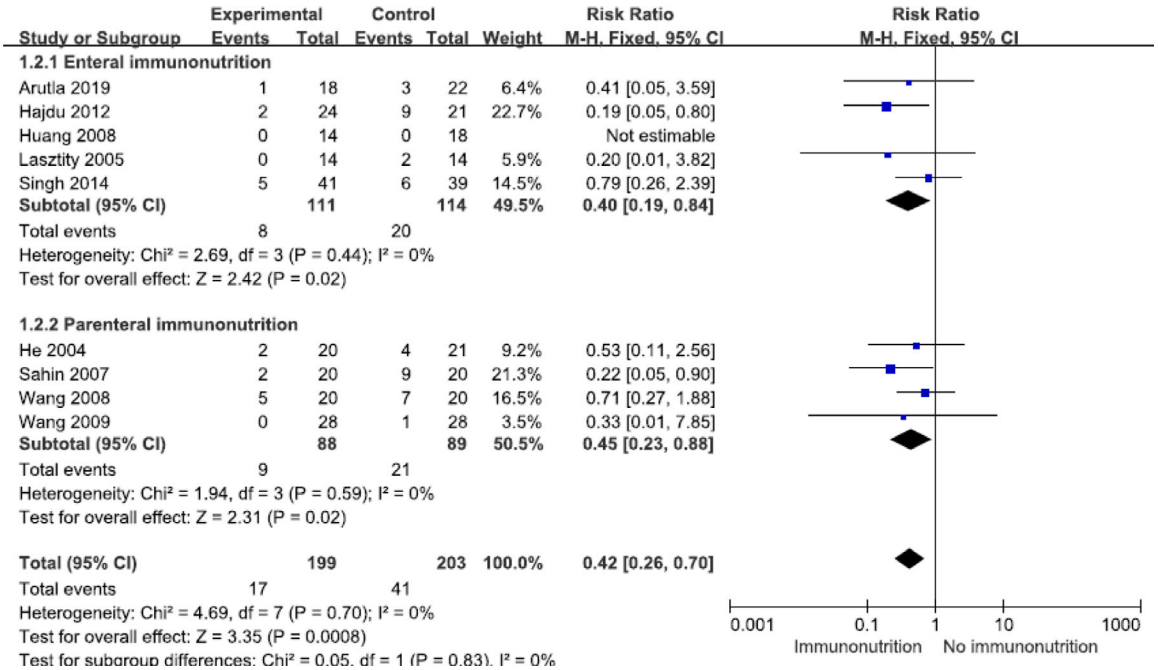

Figure 5 Forest plot of effect of immunonutrition on organ failure.

(MD = 0.88 g L^{-1}; 95% CI = −0.33 to 2.09; P = 0.15). Three RCTs with 115 patients involved the data of IL-6. There was heterogeneity between these studies (P = 0.26, I^2 = 26.0%). The results obtained showed that ImN reduced the IL-6 in patients with acute pancreatitis (MD = −14.75; 95% CI = −29.48 to −0.02; P = 0.05).

Six studies including 240 patients reported CRP. There was heterogeneity across studies ($P = 0.001$, $I^2 = 75.0\%$). The meta-analysis of RCTs showed that ImN reduced CRP in patients with acute pancreatitis (MD = −24.16; 95% CI = −48.27 to −0.05; $P = 0.05$). Subgroup analysis was conducted to reveal the effect of EIN and PIN separately. The results obtained showed that EIN did not reduced CRP significantly (MD = −16.38 days; 95% CI = −50.41 to 17.65; $P = 0.35$). PIN can reduce CRP in patients with acute pancreatitis (MD = −35.48; 95% CI = −56.40 to −14.55; $P = 0.0009$).

Discussion

In consideration of the conflicting RCT findings when comparing ImN with standard nutrition, many meta-analyses [13,33,34] have been performed aiming to determine whether the status of patients with AP may be changed by immune enhancers or not. Unfortunately, the data from these meta-analyses have failed to provide convincing evidence and most quantitative assessments assess the single part of ImN. For example, Jafari T *et al.*[33] evaluated the impact of PIN on the prognosis of patients with AP, and concluded that these formulas reduced infection and mortality, and shortened the length of hospital stay, although enteral feeding has been repeatedly proven to be superior to PN with respect to the outcomes such as nosocomial infection and even mortality rate [15,35]. Asrani V *et al.*[13] assessed the effect of glutamine supplementation and found the benefit in reducing risk of mortality and total infectious complications but not length of hospitalisation. Compared with standard EN, Petrov *et al.* [36] found that EIN was not associated with the significantly reduced risk of total infectious complications, death and duration of hospital stays. Given that there is not ample RCT or a sufficient sample size, better designed trials are recommended in patients with AP.

Our meta-analysis is the first comprehensive and dedicated evaluation of the EIN and PIN of patients with AP. In addition, the number of studies and sample size included is the largest in the appraise of immune nutrition of AP patients. Several interesting key findings were obtained in the systematic review. One of the most important findings of our study is that immune-enhanced supplementation was relevant to a significant reduced risk of mortality, which is consistent with the previous analyses [13,33,34]. At the same time, a significant difference was also found in the two main factors that affect the mortality, organ failure and infection [1], which is an interesting phenomenon that has not been reported previously.

The present study shows a reduction in organ failure and infectious complication occurrence by means of ImN, both through enteral and parenteral route. The ImN is the use of nutrients to improve nutritional status and to modulate the immune and inflammatory responses to a stress [11]. Patients who die of sepsis and multiple organ failure show evidence of immunosuppression by biochemical, flow cytometric and immunohistochemical findings [37]. Early death of immune cells may result in increased levels of circulating histones in AP, immune cells such as neutrophils may be a major contributor to histone release, either via neutrophil extracellular trap formation (i.e. NETosis) [38] or necrosis. Early plasma histone quantification of AP can predict persistent organ failure [39]. Omega-3 FAs have been shown to modulate inflammation and immune-reactions as one of the main components of the ImN formulations and may be of use with respect to immunomodulatory interventions in critically ill patients [40,41,42]. Arginine is an essential metabolic substrate for immune cells, playing an important role in normal lymphocyte function, T lymphocytes multiplication and maturation, as well as in the immune response against stress [43,44,45]. Glutamine is the most abundant amino acid in the plasma that increases the role of the lymphocytes and aids in antioxidant defences. It also facilitates bacterial translocation, decreases systemic inflammatory responses and sepsis, and is significant in critical diseases such as acute pancreatitis [46,47,48]. The early occurrence of systemic inflammatory response syndrome in severe AP patients and subsequent multiple organ dysfunction syndrome results in a high metabolism, ultimately leading to an increase in the body's demand for nutrition, with early supplemental ImN filling this demand, which may explain the lower prevalence of the ImN group regardless of the route of intervention [49].

Another finding is the shortened LOS with an increased albumin level and diminishing CRP and IL-6 levels. A shortened LOS means a shorter recovery time, which is a definite outcome followed by the former low occurrence of mortality, organ failure and infection. The probable explanation for the change of albumin, CRP and IL-6 is that they are metabolic markers that can be affected by various experimental procedures and conditions in various units, and even influenced by different races. Because we know that IL-6, CRP and albumin levels have a joint influence [50,51,52], we have illustrated the three markers in the present review. It is worth noting that ImN was found to significantly reduce the risk of total interventions, including surgery, drainage and debridement, and so on. Patients with AP who need intervention are often accompanied by complications such as bleeding or more serious illnesses [53]. BMI is one of the most easily available nutritional evaluation indicators [54,55,56]. Obesity can increase the adverse risk of prognosis in critically ill patients [57]. Some studies

have shown that obese AP patients have a worse prognosis compared to normal individuals [58,59,60]. We found that the use of immune nutrition can increase the degree of BMI decline in patients, which may indicate that immune nutrition is more beneficial to patients than prevously assumed.

Our meta-analysis, which includes all eligible existing EIN or PIN-related RCTs, not only confirmed the improvement effect of ImN on mortality, but also found a difference in the two main factors affecting it: organ failure and infection, filling the clinical gap in which EIN or PIN could improve the final prognosis of patients with AP. However, as a result of the lack of relevant research comparing the effect between EIN and PIN, there are no corresponding conclusions that can be drawn from our research. In this case, we recommend that more studies are performed with the aim of filling in the missing information.

There are certain some limitations to our meta-analysis: (i) the methodological quality of the included studies was only moderate; (ii) there were limited samples in most trials; (iii) there was a low number of studies on subjects such as arginine; (iv) there was possible heterogeneity of the seriousness of the disease in the studies as a result of the use of various severity assessments; (v) and there was an absence of detailed definitions of different concept and data on antibiotic use, which may influence the outcomes, specifically the rate of infectious complications.

In conclusion, this meta-analysis indicates that ImN is preferable to standard nutrition therapy in patients with AP. It is advised to add immune-enhanced formulas either via the enteral or parenteral route. However, as a result of the quality of the included studies, more standard RCTs are required to allow more definitive conclusions to be made regarding the effects of ImN on clinical outcomes.

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Conflict of interest, source of funding and authorship

The authors declare that they have no conflicts of interest.
No funding declared.
JZ and YX were involved in the study design, literature search, data extraction, data synthesis and the drafting of the manuscript. XKL and YL were involved in the literature search, data extraction and data synthesis. JZ and YX were involved in the data synthesis and statistical analysis. All authors were involved in assessing the quality of the articles. ZHT and WQL supervised the study. All authors critically reviewed the manuscript and approved the final version submitted for publication.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with PRISMA guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Forest plot of effect of immunonutrition on the length of stay in hospital.

Figure S2. Forest plot of effect of immunonutrition on total interventions.

Figure S3. Forest plot of effect of immunonutrition on body mass index.

Table S1. The results for the included laboratory indices.

GASTROINTESTINAL DISEASE

Exclusive enteral nutrition in the management of Crohn's disease: a qualitative exploration of experiences, challenges and enablers in adult patients

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Keywords
adherence, challenges, Crohn's Disease, enablers, exclusive enteral nutrition, patient experience.

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Abstract

Background: Exclusive enteral nutrition (EEN) is a first-line treatment for Crohn's disease in paediatrics with similar efficiency to corticosteroids. Benefits in adults have not been consistently observed with non-adherence cited as a limiting factor. This study aimed to gain an in depth understanding of the adult patient experience with EEN, identifying challenges and enablers to inform clinicians in the development of strategies to help increase patient adherence.
Methods: This exploratory, qualitative study utilised individual semi-structured interviews with 17 adult patients who had completed EEN. Participants were purposively recruited across a range of ages, social backgrounds and clinical experience. Interviews were audio recorded, transcribed and independently thematically analysed by two researchers to provide consensus in identifying key themes.
Results: Three major themes were identified. (i) Personal experience of EEN evolved over time, with the first few weeks the most challenging, becoming easier as symptoms improved, and participants became used to the EEN regimen. (ii) Participants developed coping strategies to manage challenges, including the impact on meal-based social participation and dietary restriction and monotony. (iii) Enabling factors for adherence to EEN included patient self-efficacy, health system support, supplement characteristics and access, and social support.
Conclusions: This study explored the evolving experiences of patients who completed a prescribed course of EEN. Patients described the challenges they faced, how they overcame them identifying enablers for adherence. This provides useful strategies for clinicians to integrate in their assessments and share with patients starting EEN.

Background

Crohn's disease (CD) is a chronic, relapsing and remitting inflammatory bowel condition associated with high morbidity, decreased quality of life and increased healthcare expenditure [(1)]. CD therapy aims to relieve symptoms, reduce inflammation, induce mucosal healing, prevent complications and optimise quality of life while minimising drug toxicity [(2)]. The treatment approaches for CD are complex with most medications having well documented side effects [(3,4)]. Alternative therapies that support induction and maintenance of remission without side effects are therefore desired.

There is increasing interest in the role of diet in CD management including exclusive enteral nutrition (EEN) as a management strategy [(5–9)]. EEN describes provision

of all nutrition via a liquid formula diet, typically for 6–8 weeks [10]. EEN is widely accepted as first-line steroid sparing therapy for treating paediatric CD, where it shows similar remission rates to corticosteroids [11]. The evidence for EEN efficacy in adults is weaker, with corticosteroids and biologics typically used as first-line therapy for remission in adults [12,13]. Poor adherence has been cited as one factor for EEN treatment failure in adults [13]. Difficulty tolerating prescribed volume of supplements, poor palatability, lack of multidisciplinary support, disruption to normal life, lack of guidance and experience in use of EEN amongst adult CD clinicians are thought to be contributing factors to non-compliance [9]. There is limited evidence suggesting that disease duration and severity may affect efficacy of EEN [14,15]. It is possible that in adults who have a longer disease course and more frequent complications, EEN may be less effective resulting in higher rates of EEN discontinuation in adults. The balance between efficacy, potential side effects, and patients' compliance is an important consideration in selecting therapeutic regimes and it has been suggested that if higher adherence can be achieved in adults then EEN may be a valuable treatment option [13].

Several reports have discussed patient adherence from a clinician perspective [16–19] however, there is limited research exploring the adult patients' experience using EEN as a treatment for CD. A survey exploring perceptions of patients and families treated with exclusive enteral nutrition could only report on paediatric data due to the poor response rate in the adult CD patients who were approached to participate in that study [20].

The aim of this study was to explore the experiences of adult CD patients who completed a prescribed period of EEN and identify challenges encountered and enablers that supported their adherence to treatment. An in depth understanding of this could inform clinicians to develop targeted strategies to support patient adherence to EEN, including the provision of specific recommendations in pre- commencement patient EEN education and help improve health outcomes.

Methods

Study approach and design

A qualitative descriptive approach using semi structured interviews was used to explore the patient experience of completing a prescribed period of EEN. This approach allows comprehensive summarisation, in everyday terms, of specific events experienced and described from viewpoint of participants with analysis and interpretation of the findings remaining as close to the data as possible. Thus, there was no pre-selection or manipulation of variables, and no prior commitment to any one theoretical view of a target phenomenon [21]. Ethical approval was granted by the Gold Coast Hospital and Health Service. All participants provided written informed consent.

Settings and participants

The study was conducted within the Inflammatory Bowel Disease (IBD) service of the Gold Coast Hospital and Health Service, a tertiary teaching hospital which manages approximately 600 patients with CD. Patients are given the option to commence EEN to treat active disease after discussion in a multidisciplinary team meeting. Those choosing to commence EEN receive nutritional management from a specialist IBD dietitian who provides comprehensive dietetic assessment at initial appointment, face to face or telephone review at 2–4 weeks into treatment, and a review in clinic at 6–8 weeks. Patients are prescribed polymeric formulas (1.0–2.0 kcal mL^{-1}) for oral consumption, with these available at subsidised rates through our health service. Adult patients (over 18 years) with CD who completed a prescribed period of EEN between February 2018 and February 2019 were eligible for inclusion. Participants were purposively sampled to include men and women of a range of ages and were invited for interview by post and telephone to attend interview between March and June 2019. Participants with insufficient English proficiency for interview were excluded.

Positionality of researchers

Participant recruitment, consent and interviews were conducted by a female research dietitian (RA, PhD) with training and experience in qualitative interview techniques and no knowledge of participant's clinical history outside of the study inclusion and sampling criteria. In establishing rapport prior to interview, participants were made aware of the interviewer's background as a dietitian to encourage frank discussion of the physical and emotional aspects of the CD and EEN journey. JE (IBD consultant) and RM (IBD dietitian) had provided clinical care for many of the participants, who were aware the study team included these treating clinicians.

Data collection

A draft interview guide was developed from the researchers' experience, reflections and the literature (Appendix 1). The interview guide was pilot tested for question clarity with two consumers meeting study eligibility criteria. Semi structured interviews were face-to-face in a private room

at the hospital or by telephone, with only interviewer and participant present. Interviews and lasted 16–28 min. Field notes were completed at the end of each interview to substantiate observations to allow nonverbal capturing and contextualisation of data. Interviews were audio recorded and transcribed verbatim. Transcripts were checked for accuracy against recordings but were not returned to participants for checking. Data collection and analysis occurred simultaneously, providing an iterative process responsive to participant's replies which allowed adaptation of questions with insights emerging as the study progressed [22]. Data collection continued until saturation was reached, defined by three consecutive interviews from which no new themes were identified [23]. Participant disease characteristics, demographic and social information were collected from medical records and by direct survey of participants.

Data coding and analysis

Thematic analysis followed the principles described by Braun and Clarke's six-point guide [24]. After data familiarisation through repeated reading, two researchers (RM, RA) independently open-coded transcripts to identify potential themes and patterns. After reviewing themes against coded transcripts, RA and RM discussed and reached consensus on the final themes, their names and descriptions. All themes were reviewed by the third member (JE) of the study team. Data extracts were selected to illustrate themes and subthemes. NVivo 12 (QSR International Pty Ltd) was used to facilitate data organisation, coding and analysis. An audit trail of the analysis process was maintained to help enhance credibility. Identified themes were used to develop a suggested model of service for health professionals supporting patients in the use of EEN.

Results and findings

Twenty-four patients who commenced EEN treatment during the study eligibility period were invited to participate. Seventeen (71%) of these were interviewed, with no response received from the remainder. Participants were representative of a range of demographics in age, gender, education levels, employment status and living arrangements. Participants ranged in the length of time they had CD, from those with new diagnoses to those who had been living with CD for decades. The majority of patients had clinically active CD based on CDAI with representations from those with different disease behaviours and location (Table 1).

Three broad themes, comprising nine subthemes, were identified from the participant responses (Table 2).

Table 1 Participant demographics and disease characteristics

Characteristic		Participants
Number, *n* (%)		17 (100)
Age, years		45.4 ± 12.6 (19–61)
Gender, *n* (%)	Women	8 (47)
	Men	9 (53)
Education, *n* (%)	Year 9–10	2 (13)
	Year 11–12	2 (13)
	Vocational education/ trade	7 (47)
	Bachelor's degree	4 (27)
	Missing data	2 (13)
Employment status, *n* (%)	Part time	5 (32)
	Full time	8 (50)
	Student	1 (6)
	Looking for work	1 (6)
	Retired/Not looking for work	1 (6)
	Missing data	1 (6)
Living arrangement, *n* (%)	Lives alone	3 (19)
	Lives with family	12 (75)
	Lives with friends/ flatmates	1 (6)
Body mass index, kg/m^2		26.54 ± 6.5 (19.2–45.7)
	Underweight, <18.5, *n* (%)	0 (0)
	Healthy weight, 18.5 –24.99, *n* (%)	7 (44)
	Overweight, 25– 29.99, *n* (%)	8 (50)
	Obese, >30, *n* (%)	1 (6)
	Missing Data	1 (6)
Disease duration, years		12 ± 10.1 (0–40)
Disease Behaviour, *n* (%)	B1 (non-stricturing, non-penetrating)	6 (35.3)
	B2 (stricturing)	9 (52.9)
	B3 (penetrating)	2 (11.8)
Disease Location, *n* (%)	L1 (ileal)	7 (41.1)
	L2 (colonic)	3 (17.6)
	L3 (ileocolonic)	7 (41.1)
Perianal disease, *n* (%)		7 (41.1)
Previous IBD surgery, *n* (%)		7 (41.1)
Disease activity, *n* (%) (at EEN commencement)	CDAI ≤ 150	4 (23.5)
	CDAI ≥ 150	13 (76.5)
	Mean CDAI	214 ± 77.4 (85–344)
	Mean calprotectin	538 ± 6.5 (54–1400)

Evolving personal experience of EEN

Subtheme a: Motivation to commence EEN

Distinct phases were apparent in participant descriptions of their experiences on EEN. Many indicated that the

Table 2 Themes and sub-themes from semi-structured interviews with 17 adults with Crohn's disease relating to their experience in the use of EEN

Theme	Subthemes
1) Evolving personal experience of EEN	a Motivation to commence EEN b Change over time c Weaning and post EEN
2) Coping strategies for the challenges of EEN	a Social impact b Dietary restriction and monotony
3) Enablers for adherence to EEN	a Self-efficacy b Health system support c Supplement characteristics and access d Social support

negative impact of disease symptoms on their quality of life motivated their willingness to try any strategy that might help, and this was central in their decision to commence EEN.

> I was really, really struggling and I was in a really bad place at the time, so I said to him, I said I'm willing to try anything. If it's going to take me not eating and going on a liquid diet, I will do that. I just didn't want to feel unwell anymore. (Participant 17)

Others indicated that fear of adverse disease outcomes was a major driver, particularly where the individual had previously experienced severe episodes or complications such as bowel obstruction. For some participants, the desire to limit the use of medications was a motivating factor. High levels of trust in treating health professionals were evident, with participants indicating that their recommendation of EEN must indicate this was the best option for them and would result in positive outcomes. On prompting, many participants indicated that they had conducted internet searches on EEN but placed little weight on this in their decision to commence.

> So, it was sanctioned from the hospital. Well, there was no question about it, the decision had been made so I just stuck to it..... If the doctor says, you should be on this it's better for you, then that's usually enough for me... I trust the hospital (Participant 4)

The use of EEN for treatment seemed logical to participants, with a general sense that dietary modification could help alleviate CD symptoms. Participants felt that a liquid diet would be easier for the bowel to handle.

Subtheme b: Change over time

Most participants experienced the early days of EEN as challenging. Some indicated they felt hungry, while for others, the challenge was not so much hunger as missing the sensation of eating and overcoming the expectation of needing to consume food to feel full. A few participants indicated that they also found the period towards the end of their EEN prescription difficult, with thoughts turning towards the foods they were looking forward to consuming once again. All participants indicated EEN became easier after the first one to two weeks, citing adjustment to the new meal regimen, as well as improvements in symptoms. These included better bowel motions and reduced bloating, pain and cramping, but also other aspects, such as increased intellectual clarity and energy levels.

> Not only did I feel better from my gut symptoms perspective, but I kid you not, probably about three weeks into EEN it's like suddenly my brain worked again. I'd been very, I guess just a bit erratic at work, because I'm in a really busy job, I manage a team, I travel a lot, and sometimes it can kind of overwhelm me. (Participant 7)

Subtheme c: Weaning and post EEN

The majority of participants were apprehensive about returning to their usual diet with many fearing this would result in the return of symptoms that had improved whilst they were on EEN. Most started weaning from EEN with meals they thought would cause them less symptoms. Some expressed surprise that progression of diet did not result in the symptoms they had anticipated. Interestingly, at the conclusion of the EEN period some participants chose to continue partial EN, feeling that this helped manage their symptoms. Others used the nutritional formulas to replace meals that they would otherwise have missed. Some had also made permanent dietary changes after EEN period, such as eliminating spicy foods and caffeinated drinks. Most participants indicated they would consider repeating EEN treatment if disease relapsed and would not hesitate to recommend EEN to others in similar circumstances.

> I feel really good now and if it ever came to it, if it ever came to me flaring up, I would just take it upon myself to not eat and go back on the diet for sure. (Participant 17)

Coping strategies for the challenges of EEN

Subtheme a: Social impact

The social impact of EEN stood out as a major challenge. There was some divergence evident in the strategies used by participants to cope with social activities focused around food and eating. Some felt they needed to avoid

such activities to be able to stick to EEN, while for others, the importance of being involved in these was paramount. In the latter, participants commonly described consuming their EEN supplements at the same time as others ate, sometimes modifying the serving style of the drinks to make them last longer or seem more like a meal (e.g. serving hot or freezing to eat like ice cream). Several participants indicated that while they avoided food-focused social functions during the more difficult earlier weeks of the EEN period, they found they were able to join in again at later stages.

> Your evening meal is a real big challenge, especially if you've got a big household so there's a nice smell of food in the house, everybody sitting down to eat and you're there having a shake. I found that I had to move myself away from that... I'd go and do something else, so I might watch something in a different room, I might pop out for a walk or something and, when they'd finished, I'd come back. (Participant 16)

Subtheme b: Dietary restriction and monotony

Most participants found the monotonous nature of EEN challenging. The polymeric formulas available were exclusively sweet tasting, with many participants indicating they wished other options were also available. Many missed the chewing aspects of meals, and some reported experiencing sore jaws on returning to solid food. Some participants found it helpful to vary flavours, temperatures and textures by mixing, blending, heating or freezing. The repetitive nature of the diet led some participants to develop strategies to avoid missing meals, such as setting clear routines or using prompts from others. One participant set specific flavours for breakfast, lunch and dinner and arranged bottles in rows so that a glance would indicate if the last meal had been taken.

> Monotony becomes a bit of its downfall after a period of one week or so. You just get it down as fast as you can, so to speak. (Participant 5)

Enabling factors for adherence to EEN

Subtheme a: Self-efficacy

Self-efficacy was resoundingly the most evident enabler for adherence to the EEN prescription, influencing engagement with the treatment and ability to persevere in the face of challenges encountered. Numerous participants indicated that personality traits related to conscientiousness were a reason for their success, stating that EEN 'was mentally hard' (Participant 11), 'you set your mind on what you're doing and you stick to it' (Participant 15) and that 'a lot of people aren't that strong to be able to do that sort of thing' (Participant 6). Many expressed a great sense of pride in having completed the prescribed period of EEN.

> I'm a businessman and when I have a problem, I find a solution and I get on with it. So, for my Crohn's that's my solution. (Participant 9)

Subtheme b: Health system support

The importance of support provided by health professionals and the healthcare system was evident in several aspects. Participants trusted that they were being provided with the best course of treatment and had expectations that they would see improvements in symptoms and health. Clear description of the EEN process and explanation of its intended mechanism of action contributed multiple enablers for adherence. Participants expressed confidence in the formulas providing for all their nutritional requirements. There was good understanding of the duration for EEN, the importance of the exclusive nature of the diet, and the required quantities. Participants indicated that the printed resources provided prior to commencement were very comprehensive.

> The fact that [dietitian] said she was going to ring me midway through. So, when she rings you want to be able to say, yes, I've been sticking to that. But the last thing you want to do is say, oh sorry, I couldn't stick to it and I had two days eating normal food. (Participant 4)

Participants also understood that there might be a period of adjustment when starting out, helping to set expectations for their experience. Participants regarded the ability to access further information and support from their doctors and dietitians as very valuable. This extended to being provided with biochemical marker results, identifying this as a motivator for continued adherence to the EEN prescription.

Subtheme c: Supplement characteristics and access

Accessibility of the nutritional formulas was another important enabling factor. In our service, these are subsidised, and participants found the costs acceptable. Several commented on this being equivalent or lower than their usual spending on groceries. Many also noted the ease and convenience of the ordering and delivery process.

Participants found the polymeric formulas available through the health service very palatable, although there was individual variation in preferred flavours. The

convenience aspect was also often mentioned, in terms of time savings in meal preparation.

> The taste is good to be honest. I was very - it's a very - I mean chocolate, vanilla and strawberry and just full of ice and put one of those and just drink it slowly. It's good. (Participant 9)

Subtheme d: Social support

Many participants made mention of the interest and support of family and friends as an enabler. The importance of this was emphasised by one participant noting that his wife, responsible for cooking in their household, lacked understanding of EEN and its capacity to meet all his nutritional needs, which made things difficult in the early weeks. The value of social acceptance seemed to extend to the wider sphere - after prompting, no participants recalled any difficulties with consuming EEN formulas within the workplace, or at outings with others at restaurants. Some participants found that explaining the medical reason for use of EEN to others was helpful, providing them with additional psychosocial support.

> For the most part it was pretty positive, like my flatmates would try and encourage me and they were even quite curious as to what the drinks would taste like (Participant 8)

Discussion

The use of EEN in adults has gathered renewed interest amongst IBD clinicians. Limited use in this population has been due to mixed reports of effectiveness, which has been largely attributed to poor adherence. This qualitative study explored adult patients' experiences of EEN, providing an exploration into their motivation to commence EEN, the challenges they faced, and to identify coping strategies and enablers that support adherence to EEN treatment.

Disease activity significantly impacts patient's health-related quality of life (HRQOL) with greater symptoms of depression and anxiety reported in active and severe CD [25–28]. Clinical remission correlates with improved HRQOL hence in current medical treatment of CD, the interrelationship between the two is increasingly becoming an essential consideration. Although complete food avoidance with the associated social inconvenience may impact quality of life, EEN has actually been shown to have a positive impact on HRQOL with significant improvement in dimensions such as bowel symptoms, social function, systemic symptoms and emotional status [29]. In our study, participants were willing to try any strategy that would alleviate their symptoms and improve quality of life, with many highlighting this as their main motivation for considering EEN. Seeing improvement in symptoms and increased energy levels as they progressed on EEN motivated patients to adhere or persevere with EEN even after the initial challenges.

Many participants expressed a good understanding of the potential mechanisms of action of EEN in inducing remission in CD. Patients perceive food and diet as important in their condition and often employ dietary related strategies and modifications to help manage symptoms and control inflammation [30–33]. Previous studies have found that implementation of dietary changes is often guided by the patient's anecdotal experience and research. Many patients feel that there are limited resources and insufficient dietary information and guidance provided by their treating clinicians [34]. In this study, the importance of health professionals in influencing the patient experience, and thus likely also their adherence to the EEN prescription, was evident across all the themes identified. This was apparent in providing the patient with motivation to commence EEN, setting expectations for what the experience would be like, providing clear guidelines for how to undertake the diet along with strategies for managing the challenges likely to be encountered, and being available to check in/ask questions throughout the process. Suggestions for health professionals supporting patients in the use of EEN are provided in Fig. 1. There is limited evidence to indicate the ideal frequency of dietetic contact during EEN. In one study, the dietitian and patient had fortnightly contact during the eight weeks of EEN suggesting that patients may require continuous support to adhere to EEN regimens [35]. In our study, participants reported the challenges as particularly evident in the first weeks and towards the end of EEN, suggesting this might be the optimal time to provide dietetic support. Many IBD centres have limited dietetic support and if EEN is to be offered as a viable option, adequate dietetic resourcing would need to be a key consideration.

IBD patients often report the impact of disease on family, work and other social practices. Management of diet may increase the burden of food shopping and preparation with patients needing to plan well in advance for activities [36]. Participants in this study appreciated the convenience of the supplement drinks and found EEN reduced some of these burdens. However, coping strategies were needed to manage meal-based social interactions. Strategies varied between participants, with some limiting social interactions during the entire EEN period whilst others found that this was required only during the initial phase. For some, maintaining health-related normality was particularly important and they prioritised

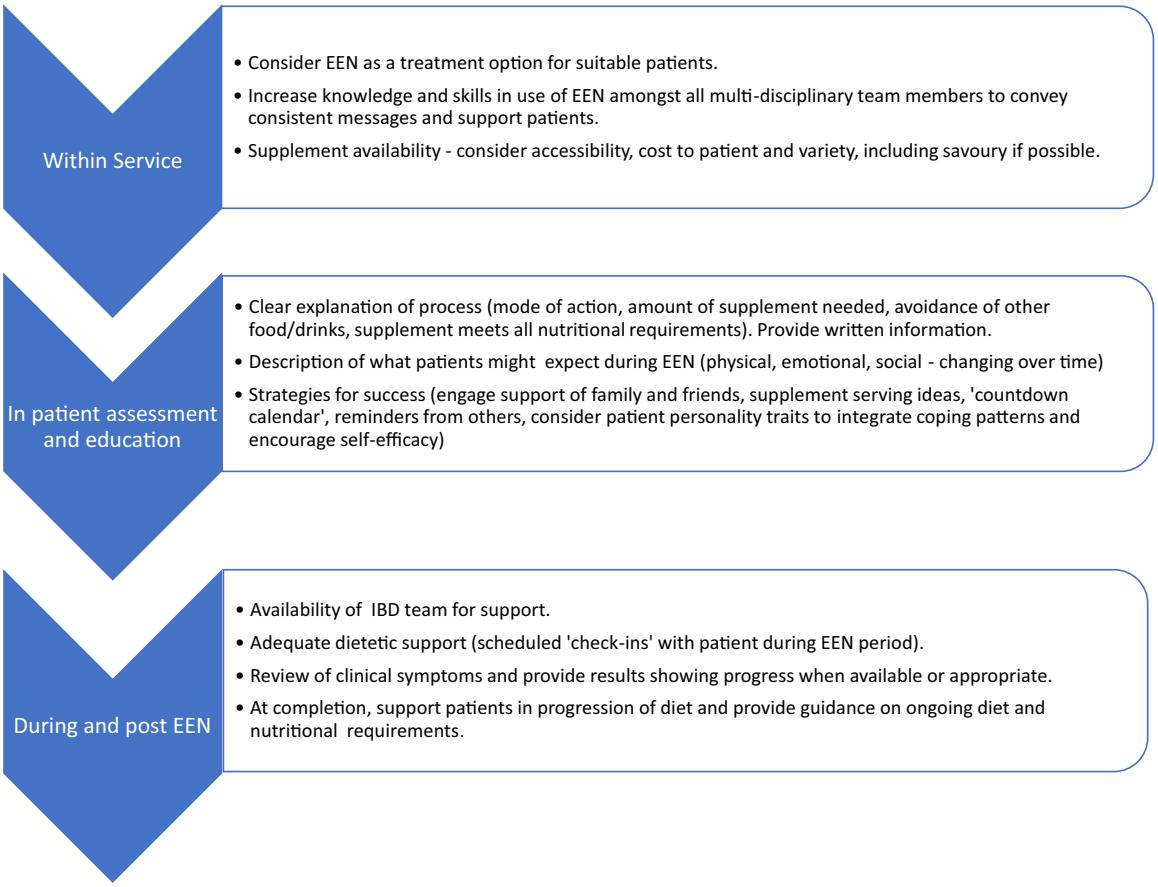

Figure 1 Strategies for health professionals supporting patients on Exclusive Enteral Nutrition (EEN).

participation in family mealtimes and restaurant visits, while maintaining adherence to EEN. This is echoed in studies that show that IBD patients continually reassess normality, fight to maintain this and often will maintain the appearance of normality to others as strategies to cope with challenges they face during the course of the condition [37]. Patients also tend to use problem-oriented coping patterns, defining problems and then generating solutions with strong emphasis on maintaining situational control [38]. In our study, this was evident in participants seeking solutions to manage their condition and addressing challenges arising during EEN with belief in their capacity to do what was required,leading us to identify self-efficacy as a key enabler for participants in adhering to the EEN prescription. Recent research has also identified an association between the conscientiousness personality trait and adherence to EEN therapy [39]. It is important for clinicians to acknowledge the social impact treatments have on patients, consider coping strategies in assessment processes and reaffirm the need for sense of normality for many patients. Referral to a psychologist, support groups or another similar resource will be dependent on the patient but may offer added value.

EEN therapy is intensive for weeks to months, hence social support may be key to adherence. Participants in this study noted that friends, colleagues, family and members of the wider community were supportive when they disclosed their diagnosis and explained use of EEN as part of their treatment. Often, patients with IBD are reluctant to disclose their condition due to lack of public awareness and perceived or anticipated stigma. This may lead to patients engaging in self-stigmatising behaviours such as avoiding social interactions [40,41]. Interestingly, no participants recalled negative experiences when they divulged their health condition or use of EEN as evidenced by the support they received, and the lack of resistance to use supplements when eating out. Patients can be encouraged to attend appointments with support persons and to form support networks whilst on EEN to help maintain compliance.

Earlier studies cited issues with cost and palatability as major barriers to effective EEN, with elemental formulas

often necessitating use of enteral tube feeding [42]. Some of these limitations have been overcome with no differences shown in remission rates for CD with use of polymeric, semi-elemental and elemental formulas [43]. The ability to use palatable oral polymeric formulations has increased the acceptability of EEN making it a realistic treatment option [41–44]. In our setting, patients are prescribed polymeric formula with these are generally well accepted, indeed taste of the supplements was highlighted by many participants as an enabler for adherence to treatment. The monotony of the diet was however a challenge for participants, but one they were able to manage by modifying supplement serving style, varying flavours or simply 'putting up with it' for the longer-term benefit. The exclusively sweet tasting nature of supplements did cause issues for some, and the recent availability of savoury polymeric formulas, occurring since the completion of this interview study, has been well received by patients trialling them in our service.

Globally, there are many variations in medical financial schemes and availability of health insurance. Consequently, in some parts of the world cost may be a significant barrier for patients in adhering to a prescription of EEN [45,46]. In our setting, nutritional formulas are provided to patients at subsided rates and many participants acknowledged that the cost of EEN was much lower than their usual food bills. Ability for patients to access affordable nutritional formulas may thus be a significant enabler assisting adherence to treatment. A systematic review found no evidence evaluating cost-effectiveness of EEN in comparison to other treatment strategies for CD [44]. Given the costly nature of some medications used, an economic analysis might be valuable given current limitations in healthcare resources.

This study has provided valuable insights into patient experiences of EEN; however, it has limitations in only enrolling. participants who had completed a prescribed period of EEN. Although this was the intent of the research team, it does not invalidate the experiences of patients who elected not to commence on EEN or those unable to adhere to recommended amounts or duration. Future studies could address this, describing the range of different patient experiences and provide further insight into barriers to EEN adherence that are not able to be overcome by some patients. Participants were recruited being recruited from a single centre with high rates of EEN use and generalisability may therefore be limited to this setting, and to patients receiving care from an IBD team experienced in the use of EEN. The use of a framework could have ensured that all aspects of potential challenges and barriers were probed, however the qualitative design of the study is a strength that provided a deeper understanding of the experiences in adults using EEN and consequently, novel insight into the factors that aid in adherence to this treatment.

Conclusion

This study provides useful strategies for clinicians to share with patients starting EEN and highlights key considerations during assessments. There is a social impact, nevertheless patients can implement strategies to cope with this and other challenges. Support from social networks and health professionals, the taste of polymeric formulas, affordability of supplements and patients' perceived self-efficacy were strong enablers for adherence. Clinicians can feel confident that adult patients are willing to consider EEN as part of management for CD and can gain emotional as well as physical benefits from this.

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Conflict of interests, source of funding and authorship

The author declared that they have no conflict of interests.
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RM, JE, RA developed the concept and study design. RA recruited and interviewed participants. RM and RA analysed data. All authors contributed to data interpretation. RA and RM drafted the manuscript. All authors reviewed and approved the final manuscript.

Ethical approval

Ethical approval was granted by the Gold Coast Hospital and Health Service Human Research Ethics Committee (LNR/2018/QGC/48492).

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with COREQ guidelines. The lead author affirms that no

important aspects of the study have been omitted and that there were no discrepancies from the study as planned.

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Appendix 1 Semi-structured interview guide: Exclusive Enteral Nutrition in the Management of Crohn's disease: A Qualitative Exploration of Experiences

Questions were not necessarily asked in the order given.

Overview of experience.

Can you give me an overview of your experience of being on EEN?

Understanding of EEN as part of treatment of Crohn's disease

Did you understand how the EEN was expected to work? Did you feel you had a clear idea of what you were required to do during EEN? What were your expectations before starting? After EEN was recommended by your doctor/dietitian, did you look for further information elsewhere? Where?

Physical experiences during use of EEN

Can you describe any physical experiences during the EEN period? If yes how did these affect you? Did this make you want to stop or want to continue with EEN?

Prompts: bowel motion changes, pain, hunger, taste; were these good or bad?

Emotional experiences during use of EEN

Do you recall any feelings or emotions you experienced during your EEN period? If yes, what were they, when did they occur?

Prompts: feeling more or less in control of condition, worries for present or future.

Factors or strategies which participants found helped them to start and continue EEN (enablers).

Did you have any or find any factors or strategies which you found helped you to take the EEN? How did they help you? Did these things change throughout the EEN period?

Prompts: Cost/access/taste of supplements, storage, information from health service/internet etc, support of others

Factors or problems which participants encountered which made EEN difficult (barriers).

What were the hardest things about being on EEN? Did these things change throughout the EEN period? Could anything be done to help overcome these issues?

Prompts: Cost/access/taste of supplements, storage, information

RENAL DISEASE

Body composition and weakness of hand grip strength and pinch strength in patients with chronic kidney disease from different ethnic backgrounds

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Keywords
appendicular muscle mass, bioimpedance, body fat mass, chronic kidney disease, hand grip strength, Sarc-F.

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Abstract

Background: Chronic kidney disease (CKD) patients commonly report muscle weakness and fatigue. Losing muscle mass increases mortality. Accordingly, we aimed to determine the main factors associated with loss of muscle mass and muscle weakness.
Methods: Anthropometric measurements were made in CKD patients attending a specialised clinic, along with hand grip strength (HGS), pinch strength (PS) and body composition (muscle mass and fat mass), using segmental bioimpedance assessment.
Results: We reviewed the results of 161 CKD patients; 105 male (65.2%), mean (SD) age 70.3 (15) years, body mass index (BMI) 28.8 (6.7) kg m^{-2}. In multivariable models, both HGS and PS were independently negatively associated with age [standardised β (St β) = 0.35; 95% confidence limits (CL) = −0.32 to −0.14; St β = 0.38; 95% CL = −0.65 to −0.02; $P < 0.001$, respectively] and positively with appendicular muscle in the arm tested [St β = 0.34; 95% CL = 2.5–6.3; St β = 0.24; 95% CL = 0.17–0.98; $P < 0.001$ and $P = 0.006$, respectively]. In addition, HGS was associated with male gender (St β = 0.19; 95% CL = 0.7–7.5; $P = 0.019$] and negatively with percentage body fat (St β = 0.22; 95% CL = −0.36 to −0.07; $P = 0.003$]. There were 47 (29.2%) Asian patients who had lower total skeletal muscle mass/height ratio and appendicular muscle mass/BMI ratio compared to other ethnicities [9.6 (1.8) versus 10.5 (1.6) kg m^{-2}, $P < 0.01$; 0.73 (0.23) versus 0.83 (0.33) m^2; $P < 0.01$).
Conclusions: In CKD patients, we found that muscle weakness measured by HGS and PS was associated with increasing age and loss of appendicular muscle mass. HGS was also weaker with increasing fat mass and female gender, whereas PS was weaker in patients of Asian ethnicity.

Introduction

Chronic kidney disease (CKD), as defined by a condition that impairs kidney function, affects more than three million UK citizens. CKD patients frequently report symptoms of muscle weakness, fatigue and muscle wasting, leading to a reduced quality of life, increased morbidity and mortality risk [1–3]. CKD patients may be potentially at greater risk of sarcopenia compared to other patient groups as a result of the retention of uraemic toxins, anaemia, CKD-bone mineral disease (CKD-BMD), vitamin D deficiency, metabolic acidosis, inflammation with increased catabolism, mitochondrial dysfunction coupled with dietary restrictions and reduced physical activity [1,4].

Clinical practice has changed over the last two decades with the availability of erythropoietin-stimulating agents

to treat anaemia, vitamin D analogues to aid the management of CKD-BMD and bicarbonate supplementation to correct metabolic acidosis. As such, we aimed to review which factors in today's clinical practice are associated with muscle weakness.

Materials and methods

CKD patients attending a specialist university hospital clinic were reviewed by a single dietician. Physical activity and muscle strength were assessed using the Sarc-F questionnaire [5]. At the same visit, anthropometric measurements were taken with respect to height, weight, triceps skin fold thickness, mid-upper arm circumference and mid-arm muscle circumference using a non-stretch tape measure and the Harpenden skinfold calliper (HSB-BI; Baty International Ltd, Burgess Hill, UK) and corrected mid-upper arm muscle area was calculated [6], along with hand grip strength (HGS) (Kern MAP 80K1, Kern & Sohn GmbH Co., Balingen, Germany) and pinch strength (PS) (Jamar Digital Plus; Lafayette Instrument Co., Lafayette, IN, USA), and body composition using bioimpedance, as part of the standard dietetic clinical assessment [7–8]. The highest value of three HGS and PS measurements were recorded.

Bioimpedance assessment measurements were made following a standardised protocol with an eight-electrode multi-frequency segmental bioimpedance device (model 720; InBody, Seoul, South Korea), which was regularly serviced and calibrated, and previously validated against dual-energy X-ray absorptiometry [9–10]. Patients with implantable cardiac devices, amputations and infected foot ulcers, and well as those with limb atrophy, were excluded.

Patient laboratory data, medications and Stoke-Davies co-morbidity were obtained from hospital computer records [11]. Sarcopenia was defined using the 2019 European Working Group on Sarcopenia in Older People (EWGSOP) and 2020 Asian Working Group for Sarcopenia (AWGS) algorithms [2,12].

Statistical analysis

Data were checked for normality, and are expressed as the mean (SD) or median (interquartile range), with comparisons made using standard statistical tests (*t* test, Mann–Whitney *U* test, analysis of variance and Kruskal–Wallis, chi-squared), with adjustments for small numbers and appropriate post-hoc testing (Tukey and Games-Howell). Univariate analysis was performed by Spearman correlation, and a multivariable regression analysis was carried out using a step backward approach, using all variables with a $P < 0.1$ correlation, with variables excluded if not statistically significant, unless they improved the model fit. Models were checked for collinearity and variable inflation factor. Analyses were conducted with standard analytical tools (PRISM, version 8.4. GraphPad Software Inc., San Diego, CA, USA; SPSS, version 25, IBM Corp., Armonk, NY, USA).

Ethical statement

Our retrospective audit of clinical practice complied with the UK National Health Service (NHS) health research authority guidelines for clinical audit and service development with all patient data anonymised (https://www.hra.nhs.uk) and approved and registered with the University Hospital.

Results

Contemporaneous data were available for 161 patients (Table 1). The majority of patients were male, and almost 50% were diabetic. Underlying renal disease was considered to be a result of diabetes 32.3%, ischaemia or hypertension in 29.8%, interstitial renal diseases in 18.6%, unclassified in 12.4% and glomerular diseases in 6.8%. The majority were of white ethnicity, followed by Asian, all but one South-Asian and then Black; three patients were of another ethnicity. The median Sarc-F score was 2, with approximately 33% of patients having an increased Sarc-F score of 4 or more. Asian patients had lower strength, both HGS and PS, compared to all other ethnicities (Fig. 1), and also had lower total skeletal muscle mass (SMM) and lower SMM adjusted for height compared to other ethnicities (Fig. 2). Appendicular muscle mass (APM) and APM adjusted for body mass index were lower in Asians compared to other ethnicities [20.8 (7.7) versus 22.5 (5.3) kg, $P < 0.05$; 0.73 (0.23) versus 0.83 (0.33) m^2, $P < 0.05$] because Asians had greater percentage of body fat [36.6% (11.2%) versus 31.6% (10.5%), $P < 0.05$].

Using the current EWGSOP and AWGS algorithms defining sarcopenia, 10% of the African patients fulfilled all criteria compared to no patients from the other ethnic groups.

On univariate regression, HGS and PS were positively associated with muscle mass in the dominant arm, skeletal muscle mass, appendicular muscle mass, male gender, haemoglobin, serum albumin and creatinine, as well as negatively associated with age, albumin, Sarc-F score and body fat (Table 2). Neither were associated with eGFR ($r = -0.03$, $P = 0.19$ and $r = 0.00$, $P = 0.98$ respectively).

In a multivariable regression model, HGS was independently associated with muscle mass in the dominant arm and male gender and negatively with age and percentage

Table 1 Chronic kidney disease patient demographics

	All	White	Black	Asian
Number	161	81 (50.3)	30 (18.6)	47 (29.2)
Male (%)	105 (65.2)	57 (70.4)	16 (53.3)	30 (63.8)
Age (years)	70.3 (15.0)	72.1 (14.2)	70.0 (14.0)	68.4 (15.0)
Weight (kg)	78.2 (19.2)	79.8 (19.0)	80.9 (19.9)	75.6 (13.1)
BMI (kg m^{-2})	28.8 (6.7)	28.7 (6.9)	29.8 (7.4)	29.0 (5.8)
Diabetic (%)	80 (49.7)	40 (49.4)	14 (46.7)	24 (51.1)
Co-morbidity	2 (1–2)	2 (1–2)	2 (1–2)	2 (1–3)
Sarc-F	2 (0–5)	2.0 (0–3.5)	2.5 (0–6)	3.0 (0–5)
Sarc-F $\geq$ 4	52 (32.3)	20 (24.7)	14 (46.7)	16 (34.0)
MAC (cm)	31.1 (5.5)	31.1 (5.5)	31.3 (5.8)	31.3 (5.6)
MAMC (cm)	20.6 (4.8)	20.9 (4.8)	20.4 (5.0)	20.3 (4.9)
TSFT (mm)	33.7 (12.9)	32.3 (12.3)	34.7 (7.0)	35.0 (11.9)
CUAMC (cm)	38.8 (17.8)	35.6 (18.8)	34.3 (16.8)	33.2 (18.1)
SMM (kg)	27.9 (6.5)	29.2 (6.1)**	29.8 (6.1)*	25.3 (6.4)
SMMI (kg m^{-2})	10.2 (1.7)	10.4 (1.6)	10.8 (1.6)*	9.6 (1.8)
APMM (kg)	22.0 (6.2)	22.9 (5.3)	22.4 (4.9)	20.8 (7.7)
APMMI (kg m^{-2})	8.0 (1.7)	8.1 (1.4)	8.2 (1.5)	7.8 (2.1)
Arm muscle (kg)	2.93 (0.79)	3.06 (0.78)	3.02 (0.66)	2.73 (0.8)
Fat mass (kg)	26.6 (12.5)	25.5 (11.4)	27.0 (14.5)	28,7 (13.1)
Fat mass (%)	33.1 (10.9)	31.5 (19)	31.6 (12.9)	36.5 (11.2)
HGS (kg)	25.0 (10.9)	26.5 (11.6)	26.5 (11.3)	21.8 (9.1)
PS (kg)	5.0 (2.1)	5.4 (2.3)**	5.1 (2.0)	4.3 (1.6)
Haemoglobin (g L^{-1})	112.9 (14.6)	114.4 (13.8)	113.7 (13.5)	111.9 (13.0)
Urea (mmol L^{-1})	20.8 (6.8)	21.9 (6.7)	20.5 (7.3)	20.0 (7.3)
Creatinine (μmol L^{-1})	315 (244–415)	306 (243–377)	344 (249–477)	284 (208–463)
eGFR (mL min^{-1} 1.73 m^{-2})	16 (12.5–21)	17 (14–21)	14 (12–17)	17 (10–23)
Albumin (g L^{-1})	42.3 (3.9)	43 (4)**	42 (3)	41 (4)
CRP (mg L^{-1})	4 (1–7)	4 (1–7)	4 (1–7)	3 (1–8)
Bicarbonate (mmol L^{-1})	22.3 (3.1)	22 (3)	22 (3)	22 (3)
Calcium (mmol L^{-1})	2.37 (0.14)	2.35 (0.12)	2.39 (0.12)	2.39 (0.14)
Phosphate (mmol L^{-1})	1.35 (0.29)	1.32 (0.25)	1.32 (0.28)	1.42 (0.35)
PTH (pmol L^{-1})	14.1 (7.4–21.7)	12.2 (6.7–21.3)	18 (9.7–26.6)	14.1 (7.3–28)
Vitamin D3 (nmol L^{-1})	69 (35–98)	61 (27–93)	78 (56–129)	56 (28–96)
VitD3 medication (%)	98 (60.9)	50 (61.7)	20 (66.7)	26 (55.3)

Patients divided by ethnicity. BMI, body mass index; MAC, mid-arm circumference; MAMC, mid-arm muscle circumference; TFST, triceps skin fold thickness; CUAMC, corrected upper arm muscle circumference; SMM, skeletal muscle mass; APMM, appendicular muscle mass; HGS, hand grip strength; PS, pinch strength; eGFR, estimated glomerular filtration rate; CRP C-reactive protein; PTH, parathyroid hormone; VitD3 medication, vitamin D3 prescription. Values are expressed as integer, percentage, mean (SD), median (interquartile range).
*$P < 0.05$.
**$P < 0.001$ versus Asian.

body fat (Table 3). PS was again independently associated not only with muscle mass in the dominant arm, but also Asian ethnicity, and was negatively associated with age.

Discussion

The body has fat reserves that can be mobilised, whereas there is no equivalent protein store. As such, skeletal muscle, which accounts for around 50% of total body protein, is the major physiological reserve and, if proteins or amino acids are required, then skeletal muscle is broken down [1]. As individuals age after their mid-30s, then muscle mass tends to be lost. The term sarcopenia was first introduced to differentiate this normal physiological loss of muscle from an accelerated or pathological loss of muscle mass [13] Using the Sarc-F screening questionnaire, approximately one-third of our patients had significantly sufficiently high scores to warrant further investigation for sarcopenia [2,5,12]. There have been debates about the relevance of measuring muscle mass in patients with muscle weakness because infiltration of muscle with fat could potentially maintain muscle bulk

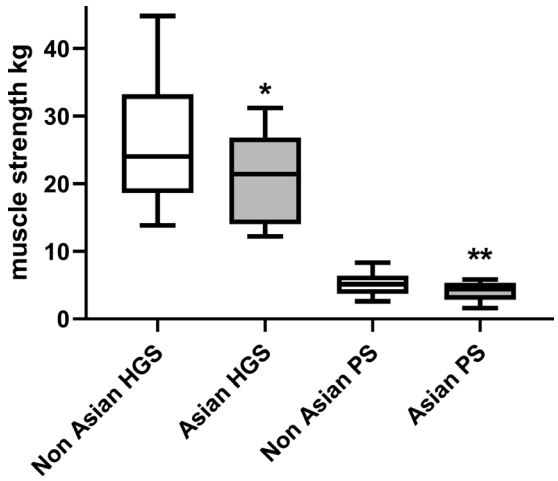

Figure 1 Hand grip strength (HGS) and pinch gauge strength (PS) in Asian patients and other ethnicities. *$P < 0.05$, **$P < 0.01$ versus other ethnicities. Date are expressed as median, interquartile and 95% confidence limits.

Table 2 Statistically significant univariate associations with Hand grip strength (HGS) and pinch gauge strength (PS)

	HGS		PS	
Variable	*r*	*P*	*r*	*P*
Skeletal muscle mass (kg)	0.60	<0.001	0.40	<0.001
Dominant arm muscle (kg)	0.55	<0.001	0.35	<0.001
% Body fat	−0.51	<0.001	−0.38	<0.001
Appendicular muscle mass (kg)	0.51	<0.001	0.35	<0.001
Male gender	0.39	<0.001	0.25	0.001
Sarc-F score	−0.37	<0.001	−0.33	<0.001
Age years	−0.33	<0.001	−0.24	0.002
Fat mass (kg)	−0.28	0.001	−0.26	0.002
mid-upper arm muscle area	0.27	0.001	0.11	0.15
Weight (kg)	0.25	0.002	0.13	0.09
Haemoglobin (g L^{-1})	0.19	0.017	0.06	0.46
Serum albumin (g L^{-1})	0.18	0.026	0.19	0.02
Ethnicity versus Asian	−0.18	0.024	−0.22	0.006
Serum creatinine (μmol L^{-1})	0.16	0.043	0.08	0.31
Triceps skin fold thickness	0.16	0.048	0.14	0.07

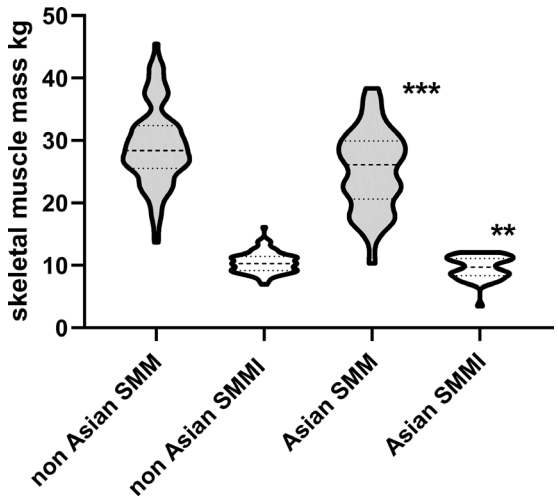

Figure 2 Skeletal muscle mass (SMM) and SMM indexed for height (SMMI) in Asian patients and other ethnicities. *$P < 0.05$, **$P < 0.01$ versus other ethnicities. Date are expressed as median, interquartile and 95% confidence limits.

[14]. However, although, on univariate analysis, we found that there was a negative association between both HGS and PS with measures of body fat, the strongest positive associations with HGS and PS in our patients were with total body skeletal muscle mass, muscle mass in the dominant arm and total appendicular muscle mass. Compared to earlier studies that used single or multiple frequency bioimpedance devices measuring whole body muscle mass, we were able to measure segmental muscle mass in the arms and legs [15–17]. However, we did not find an association between assessments of muscle mass in the arm based on standard anthropometric methods.

Appendicular muscle mass was relatively well maintained, with very few of our patients having sarcopenia according to current clinical guideline definitions [2,3,12,18], whereas muscle strength was reduced, particularly in our male patients across ethnic groups, compared to studies reporting on age equivalent healthy patients [19–21]. The loss of muscle strength with loss of muscle mass may be a result of increased catabolism, as associated with inflammation and metabolic acidosis [1,22]. However, we found no relationship between HGS or PS and serum C-reactive protein or bicarbonate, although there was an association with serum albumin. Previous studies have commented on a lower serum albumin in patients with CKD [4], although whether this reflects reduced nutritional status [23], or is more a marker of inflammation, remains a matter of debate [24].

In addition to muscle weakness, patients with CKD typically report fatigue. Muscle fatigue could be exacerbated by uraemic solutes, anaemia or vitamin D deficiency [14,22]. In the present study, we observed no effect of serum urea, creatinine, estimated renal glomerular filtration rate, stage of CKD, vitamin D levels, or prescription of vitamin D3, or parathyroid hormone, on HGS or PS.

Patients with CKD have been reported to have reduced active energy expenditure [25], and exercise programmes have been reported to increase muscle strength [26,27], reduce fatigue and improve quality of life [28]. The Sarc-F questionnaire provides some information about physical

Table 3 Multivariable step backward regression model for Hand grip strength (HGS) and pinch gauge strength (PS)

Variable	β	SE-β	ST-β	*t*	95% CL	Col	VIF	*P*
HGS	model							
Age year	−0.23	0.05	−0.35	−5.1	−0.32 to − 0.14	0.74	1.3	<0.001
Arm muscle kg	4.4	0.98	0.34	4.5	2.5 to 6.3	0.65	1.6	<0.001
% Fat	−0.22	0.07	−0.22	−3.0	−0.36 to − 0.07	0.68	0.5	0.003
male	4.1	1.7	0.19	2.4	0.7 to 7.5	0.56	1.8	0.019
ethnicity	−1.4	0.75	−0.13	−1.9	−2.9 to 0.08	0.78	1.3	0.06
Albumin (g L^{-1})	0.29	0.16	0.11	1.8	−0.03 to 0.62	0.94	1.1	0.075
PS	model							
Age year	−0.04	0.01	−0.38	−4.0	−0.65 to − 0.02	0.94	1.1	<0.001
ethnicity	−0.61	0.18	−3.0	−3.5	−0.96 to − 0.27	0.9	1.1	0.001
Arm muscle (kg kg^{-1})	0.58	0.21	0.24	2.8	0.17 to 0.98	0.9	1.1	0.006

r, Spearman rho. Arm muscle – dominant arm, ethnicity versus white, unstandardised beta (β), standard error β (SE-β), standardised β (ST-β), 95% confidence limits (95% CL), collinearity tolerance (Col) and variable inflation factor (VIF). Model for HGS Nagelkerke, unadjusted r^2 0.577, adjusted r^2 = 0.556 and, for PS, r^2 = 0.29 and 0.27 respectively.

fitness and there was a univariate association between muscle strength and lower Sarc-F scores, whereas there was no such association with co-morbidity scores.

We noted that patients from an Asian background had lower muscle mass compared to white and black patients and had a lower appendicular muscle mass compared to other ethnicities [12–29]. This supports previous reports conducted in kidney dialysis patients and has been recognised by guideline committees that have proposed different parameters for defining sarcopenia in Asian patients compared to Europeans [2,12,18]. Not only is muscle mass lost with age, but also there is often an increase in truncal fat as people age, and this has led to the concept of sarcopenic obesity [14], with our Asian patients demonstrating a higher appendicular muscle mass to body mass index ratio compared to other ethnicities.

We report on a cohort of CKD patients attending a specialist CKD clinic designed to prepare patients for dialysis, pre-emptive transplantation or conservative care. As such, our patients were receiving treatment for anaemia, metabolic acidosis, CKD-BMD and cardiovascular risk factors, including blood pressure and fluid management. Unlike previous studies that reported only a weak statistical association between muscle mass and HGS in CKD patients [30], suggesting a divergence between muscle mass and function, by using segmental bioimpedance, we demonstrated a very much stronger association between measurements of limb muscle mass and muscle strength.

Although there are many potential causes explaining why CKD patients may be at greater risk of muscle weakness and fatigue, ranging from anaemia to metabolic causes, including loss of renal function with retention of uraemic toxins, acidosis, vitamin D deficiency, hyperparathyroidism, inflammation with increased catabolism, as well as reduced physical activity, we found that loss of muscle strength as assessed by HGS was independently associated with increasing age and body fat, female gender and loss of muscle in the arm. Similarly, weak PS was associated not only with increasing age and loss of muscle mass in the arm, but also Asian ethnicity. As such, in the modern era, by treating anaemia with erythropoietin stimulating agents, correcting metabolic acidosis and treating vitamin D deficiency, the main causes of muscle weakness in CKD patients are age, as well as the associates of age, loss of appendicular muscle and gain in body fat.

Conflict of interests, source of funding and authorship

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All authors critically reviewed the manuscript and approved the final version submitted for publication. KJ made measurements, analysed data and contributed to writing manuscript. AS devised project, provided equipment and contributed to writing manuscript. AD devised project, obtained study approvals provided equipment and contributed to writing manuscript.

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NUTRIENT GENE INTERACTIONS

Leptin gene polymorphism (rs 7799039;G2548A) is associated with changes in lipid profile during a partial meal-replacement hypocaloric diet

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Abstract

Background: Some studies have demonstrated a positive association of the rs7799039 genetic variant of the *LEP* gene with energy intake and metabolic parameters. The present study aimed to analyse the effects of the rs7799039 genetic variant of the *LEP* gene on metabolic parameters after weight loss secondary to a partial meal-replacement (pMR) hypocaloric diet.
Methods: We conducted a non-randomised, single-treatment study in 122 obese subjects with body mass index (BMI) > 35 kg m^{-2}. The subjects were treated with two intakes of a normocaloric hyperproteic formula during 12 weeks. Anthropometric parameters and biochemical profile were measured at basal time and after 12 weeks. The variant genetic variant (rs7799039) of the *LEP* gene was assessed by a real-time polymerase chain reaction.
Results: We recruited 122 subjects [26 GG (21.3%), 59 GA (29.5%) and 37 AA (30.3%)]. The mean (SD) age of the all group was 59.4 (6.3) years (range 45–63 years) and the mean (SD) BMI was 39.3 (2.8) kg m^{-2} (range 36.2–45.1 kg m^{-2}). After the pMR hypocaloric diet, body weight, BMI, fat mass, waist circumference, fasting insulin, homeostasis model assessment for insulin resistance and blood pressure decreased in both genotypes. All of these improvements were similar in both genotypes. Moreover, after dietary intervention, only subjects without an A allele showed a significant improvement in triglycerides (GG versus GA + AA) [mean (SD) −15.3 (6.4) mg dL^{-1} versus −3.7 (4.3) mg dL^{-1}: $P = 0.02$], total cholesterol [−25.0 (5.3) mg dL^{-1} versus −8.1 (3.5) mg dL^{-1}: $P = 0.02$] and low-density lipoprotein-cholesterol [−20.7 (4.2) mg dL^{-1} versus −5.4 (2.3) mg dL^{-1}: $P = 0.01$].
Conclusions: Subjects with an A allele of the rs7799039 variant in the *LEPR* gene showed a significant improvement in low-density lipoprotein-cholesterol and triglycerides levels after weight loss secondary to a pMR hypocaloric diet.

Introduction

Obesity is one of the main causes of mortality and morbidity, including cardiovascular events, diabetes mellitus type 2 and cancer. In recent studies, the prevalence of obesity in the world is reported to be >10% [(1)] and, in Spain in particular, it is 22% [(2)]. The main objective in these patients with respect to reducing associated morbidity is weight loss. The cornerstone of these treatments includes a reduced-calorie diet with exercise, with the goal of achieving a weight loss of at least 5–10% in a short-term period [(2)]. One option for this reduction of

calories comprises a partial meal-replacement (pMR) diet. Heymsfield *et al.* [3] performed a meta-analysis comparing partial replacement diets wityh traditional energy-restricted food-based diets and demonstrated that pMR diets produced superior weight loss than conventional diets: 7% versus 3% in 3 months.

This healthy weight loss in response to diet interventions showed significant inter-individual variability related to the genetic background [4]. The genes involved in the leptin pathway are implied to affect weight loss and secondary metabolic changes. Leptin is a cytokine synthesised in white adipose tissue (16 kDa peptide) and has different roles in regulating body weight. The gene for leptin is located at chromosome 7q31.3, encodes a 3.5-kb cDNA, and has three exons and two introns. Some single nucleotide polymorphisms (SNPs) of gene for leptin have shown their functionality in the promoter region (rs7799039; −258G/A) [5]. The A allele was reported to lead higher mRNA expression and leptin plasma levels [6]. Some studies have demonstrated a positive association of this SNP of the *LEP* gene with energy intake [7,8]. One of them showed that subjects carrying the AA genotype had a higher energy intake [7], whereas the other reported that pregnant women with the A allele had a lower energy intake than non-A-carriers [8]. Leptin is a satiety hormone that acts on anorexigenic neurones and gives signals to the hypothalamus, increasing energy expenditure and decreasing appetite [9]. Taking into account all the previously mentioned data, there may be a relationship between this SNP and the loss of weight or metabolic changes induced by low-calorie diets. However, to date, no study has evaluated the role of rs7799039 in metabolic changes secondary to a pMR diet.

The present study aimed to analyse the effects of rs7799039 genetic variant of the *LEP* gene on metabolic parameters after weight loss secondary to pMR hypocaloric diet.

Materials and methods

Study design

This interventional trial was conducted at a Public Hospital in Spain from January 2018 to January 2020. All participants provided their written informed consent. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (HVUVA committee 2/2018) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The design of the study was a single-treatment study, non-randomised, with a pMR hypocaloric diet with a normocaloric hyperproteic formula.

The enrollment of 122 obese patients was achieved by a consecutive method of sampling from Primary Care patients. Obese subjects were invited to participate in the study if they met the following eligibility criteria: aged between 40 and 65 years, with body mass index (BMI) > 35 kg m^{-2}. The exclusions included: uncontrolled thyroid disease, previous cardiovascular events (heart attack or ictus), severe renal or hepatic disease, active alcoholism, malignant tumor, or receiving medications known to influence lipid or glucose levels within 6 months before the start of the study.

Anthropometric parameters (weight, height, body mass index (BMI), waist circumference and fat mass by impedance) and blood pressure at basal time and after 12 weeks were measured for all enrolled subjects. A trained professional collected fasting blood samples into tubes containing ethylenediaminetetraacetic acid, at baseline and at 12 weeks, for analysis of insulin, total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol and triglyceride levels. The variant of the *LEP* gene was assessed by a polymerase chain reaction (PCR).

Dietary intervention

After enrolling patients into the study and obtaining their informed consent, they received nutritional education to complete a pMR hypocaloric diet. This pMR was achieved with six meals (breakfast, morning snack, lunch, afternoon snack, dinner, after dinner snack). The lunch and dinner were replaced by an artificial nutritional preparation (VEGEStart Complete®; Vegenat Healthcare S.L., Badajoz, Spain), for which the nutritional characteristics are described in Table 1 (normocaloric hyperproteic formula). A dietitian made a reinforcement telephone call every 7 days and all patients completed a dietary record over 72 h to assess the intake of calories and macronutrients, at baseline and after 12 weeks. The records were analysed using DIETSOURCE (Nestlé, Geneva, Switzerland). The physical activity recommendations for patients of both groups were the completion of aerobic physical activities at least three times per week (60 min each). The exercises that were indicated included walking, running, cycling, swimming and muscular strength exercises (weight training or weightlifting). The exercise activity of patients was self-reported via a questionnaire.

Anthropometric parameters and blood pressure

Anthropometric measures were obtained in accordance with standardised techniques by a trained dietitian. Height was determined with the patient in an upright, using a stadiometer (Seca, Birmingham, UK). Body weight was determined without clothing to an accuracy of ±0.1 kg, using a manual scale (Seca). BMI was calculated as: weight (kg)/height × height (m^2). The difference

Table 1 Nutrient composition of partial meal-replacement diet (three intakes as natural food and two intakes as artificial formula)

Parameters	Diet + Formula	Normocaloric hyperproteic formula (200 mL)
Caloric value (kcal)	1035	200
Proteins [g (% TCV)]	64.4 (25%)	15.4 (31%)
Lipids [g (%TCV)]	19.1 (17%)	5.2 (23%)
Carbohydrates [g (%TCV)]	151.6 (59%)	21 (42%)
Fibre (g)	15.9	4.2

Normocaloric hyperproteic formula is VEGEstart® (%TCV, total caloric value percentage).

in relative weight was determined by the percentage of weight loss (%PP) as: [weight before intervention − weight after intervention (kg)/initial weight (kg)] × 100. Waist circumference was measured in the narrowest diameter between the xiphoid process and the iliac crest. A bioelectrical impedance analysis (BIA) was also conducted. An alternating current of 0.8 mA at 50 kHz produced by a calibrated signal generator (EFG; Akern, Firenze, Italy) was used and applied to the skin via adhesive electrodes placed on the back of hand and right foot, after a fast of at least 8 h. The parameter analysed with the BIA was total fat mass (kg) [10].

Finally, blood pressure was measured three times after 10 min of rest with a random zero mercury sphygmomanometer and then averaged (Omrom, Los Angeles, CA, USA).

Biochemical parameters

Glucose metabolism was measured with fasting glucose, fasting insulin and homeostasis model assessment for insulin resistance (HOMA-IR). Insulin was measured by radio-immunoassays (RIA Diagnostic Corporation, Los Angeles, CA, USA) with a sensitivity of 0.5 mUI L^{-1} (normal range 0.5–30 mUI L^{-1}) [11], fasting glucose levels were analysed by using an automated glucose oxidase method (Glucose analyser 2; Beckman Instruments, Brea, CA, USA) and then HOMA-IR was calculated using these values [12].

The lipid profile was measured and included total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides. Total cholesterol and triglyceride concentrations were measured using an enzymatic colorimetric technique (Technicon Instruments, Ltd, New York, NY, USA). HDL was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulphate-magnesium. LDL-cholesterol was calculated using the Friedewald formula (LDL-cholesterol = total cholesterol − HDL-cholesterol − triglycerides/5) [13].

The DNA was extracted using a commercial kit as indicated by manufactured (Applied Biosystems, Foster City, CA, USA) from oral mucosa cells. Genotyping (rs7799039) was performed using customised assays with the TaqMan® OpenArray™ Genotyping platform (Thermo Fisher, Pittsburg, PA, USA). Samples were loaded using the AccuFill system (Thermo Fisher) and amplification was carried out using a QuantStudio 12K Flex Real-Time qPCR instrument (Thermo Fisher). A total volume of 10 μL with 2.5 μL of TaqMan OpenArray Master Mix (Applied Biosystems) and 2.5 μL of human DNA sample were loaded and amplified on arrays in accordance with the manufacturer's instructions. Genotype calling and sample clustering for OpenArray assays was performed using a TaqMan Genotyper (Life Technologies, Carlsbad, CA, USA).

Statistical analysis

Statistical analysis was conducted using SPSS, version 23.0 (IBM Corp., Armonk, NY, USA). The genotype distribution was evaluated for deviation from Hardy–Weinberg equilibrium by chi-squared. The variant of the *LEP* gene was in Hardy–Weinberg equilibrium ($P = 0.33$). A sample size was calculated to detect differences over 10 mg dL^{-1} for cholesterol with 90% power and 5% significance ($n = 120$). All biochemical and anthropometric parameters were examined for normality with the Kolmogorov–Smirnov test. All results are expressed as the mean (SD). In within-groups, we used a paired Student's *t*-test for biochemical parameters at baseline and after 12 weeks of intervention. In between-groups, an independent *t*-test was used to compare differences. The Mann–Whitney *U*-test was used for non-parametric variables. Categorical variables were analysed with a chi-squared test, with Yates correction as necessary, and Fisher's test. Univariate analysis of covariance with a post-hoc Bonferroni test was used to evaluate the gene–diet interaction. The statistical analysis was performed as a dominant model, for the combined *GA* and *AA* as a group and with *GG* genotype as second group (wild-type genotype). $P < 0.05$ was considered statistically significant.

Results

We recruited 122 subjects [26 GG (21.3%), 59 GA (29.5%) and 37 AA (30.3%)]. All patients fulfilled the 12-week follow-up period without dropouts and no adverse events related to intervention were detected. The mean (SD) age of the all group was 59.4 (6.3) years (range 45–63 years) and the mean (SD) BMI was 39.3 (2.8) kg m^{-2} (range 36.2–45.1 kg m^{-2}). The sex distribution comprised 36 males (29.5%) and 86 females (70.5%). The sex

distribution was similar in genotype groups: males (32.0% versus 28.1%) and females (68.0% versus 71.9%). The mean age was similar in both genotype groups [wild-type (GG) versus mutant type (GA + AA)] [60.1 (7.1) years versus 58.3 (5.0) years; not significant].

In this intervention trial, obese subjects with the GG genotype showed a decrease in calorie and macronutrients (carbohydrate, fat and protein). These improvements (Table 2) were statistically significant with respect to calories day^{-1} [Δ 528.1 (34.7) mg dL^{-1}; $P = 0.02$], carbohydrates [Δ 37.1 (4.7) mg dL^{-1}; $P = 0.01$], lipids [Δ 23.7 (6.8) mg dL^{-1}; $P = 0.02$] and proteins [Δ 21.1 (2.2) mg dL^{-1}; $P = 0.01$]. The distribution of fats comprised 32.7% saturated fats, 50.2% monounsaturated fats and 17.1% polyunsaturated fats. Physical activity was similar at both times (119.2 (22.3) versus 130.1 (28.2) min $week^{-1}$: $P = 0.71$).

The same profile of basal dietary intakes and post-treatment intakes was reported in the A allele carriers. Patients with GG genotype also showed a decrease in calorie and macronutrients (carbohydrate, fat and protein). These improvements (Table 2) were statistically significant with respect to calories per day [Δ 533.1 (28.1) mg dL^{-1}; $P = 0.02$], carbohydrates [Δ 26.1 (4.3) mg dL^{-1}; $P = 0.01$], lipids [Δ 39.1 (5.8) mg dL^{-1}; $P = 0.01$] and proteins [Δ 15.1 (2.7) mg dL^{-1}; $P = 0.01$]. The distribution of fats comprised 32.3% saturated fats, 50.3% monounsaturated fats and 17.4% polyunsaturated fats. Physical activity was similar at both times [121.2 (17.3) versus 131.2 (21.2) min $week^{-1}$; $P = 0.65$]. Basal and post-dietary intakes were similar in both genotypes. As indicated by all of the results outlined above, there was a significant decrease after the caloric intake intervention and for all macronutrients except fibre intake.

As reported in Table 3, the anthropometric parameters and blood pressure parameters were similar in both genotypes at baseline. After the pMR hypocaloric diet, body weight, BMI, fat mass, waist circumference and blood pressure decreased in both genotypes. All of the improvements in these parameters were similar in both genotypes. The percentage of weight reduction at 12 weeks was 7.7% (5.1–9.8%) in non-A allele carriers and 7.8% (6.0–9.2%) in A allele carriers without statistical differences.

Table 4 shows the biochemical parameters. Fasting insulin levels and HOMA-IR improved in both genotypes. Moreover, after dietary intervention with the meal-replacement hypocaloric diet, only subjects without A allele showed a significant improvement in triglycerides GG versus GA + AA) [−15.3 (6.4) mg dL^{-1} versus −3.7 (4.3) mg dL^{-1}: $P = 0.02$], total cholesterol [−25.0 (5.3) mg dL^{-1} versus −8.1 (3.5) mg dL^{-1}: $P = 0.02$] and LDL-cholesterol [−20.7 (4.2) mg dL^{-1} versus −5.4 (2.3) mg dL^{-1}: $P = 0.01$].

Discussion

The main finding of our interventional study was that individuals without the A allele of SNP rs7799039 showed a significantly greater improvement of LDL-cholesterol and triglycerides than A allele carriers after a pMR hypocaloric diet. Patients of both genotypes showed a significant improvement in anthropometric parameters, blood pressure and insulin resistance.

One of the first findings of our study is the lack of relationship of this polymorphism with caloric or macronutrient intake. In the literature, there is controversy regarding this relationship. A case–control study with Arabic patients showed that adults carrying the AA genotype had significantly higher daily energy intake [GG: 2853 (1215) kcal versus GA + AA: 3431 (1609) kcal] [7]. Moreover, in a cohort study [13] among Brazilian pregnant females, a significant association between allele A of rs7799039 and the change in energy intake from prepregnancy to pregnancy was reported, with carriers of the A allele having lower total mean adjusted energy intake (GA + AA = 1964 kcal day^{-1} versus GG = 2192 kcal day^{-1}). However, a randomised intervention trial in Caucasian men reported no effect of the A allele of the LEP-rs7799039 polymorphism on *ad libitum* energy intake [14]. This lack of association was also demonstrated in another study with a Caucasian population [15] and in a prospective cohort of Brazilian children [16]. Finally, in a randomised cross-over trial in overweight subjects, Douglas *et al.* [14] reported that the A allele is related to the variation in the feeling of fullness and regulation of food intake. However, this 1-week-long study did not evaluate the effect on total energy intake.

The rs7799039 polymorphism consists of a guanine to adenine substitution in the promoter region [7] and this mutation could affect gene expression, probably at the transcriptional level, modifying the levels of leptin produced by adipose tissue and consequently affecting metabolic parameters and satiety control [17,18]. However, the findings of studies investigating the risk allele are controversial [13-16] with respect to body weight and there are few studies available in relation to the metabolic or secondary aspects of a nutritional intervention. For example, some treatments that produce weight gain, such as antipsychotic drugs, have been related to this polymorphism. In a meta-analysis by ethnicity, Shen *et al.* [19] have reported that the rs7799039 polymorphism was associated with antipsychotic-induced weight gain in an Asian population. Moreover, in European populations, the A allele appeared to decrease the risk of weight gain secondary to these drugs. The explanations for these controversial findings on body weight may be that, first, most of these studies were designed with a small size with an increased risk of a type I error. Second, the patients took different types of

Table 2 Dietary intakes at baseline and after 12 weeks [mean (SD)]

	n = 122					
	GG (*n* = 26)			GA + AA (*n* = 96)		
Parameters	Basal	3 months	*P* Time Genotype Genotype × time	Basal	3 months	*P* Time Genotype Genotype × time
Calorie intake (cal day^{-1})	1617.2 (231.8)	1007.9 (99.1)*	*P* = 0.02 *P* = 0.32 *P* = 0.03	1540.1 (191.8)	1001.1 (82.1)*	*P* = 0.02 *P* = 0.33 *P* = 0.03
Carbohydrate intake (g day^{-1}) (PTC %)	169.1 (53.9) (39.6%)	132.1 (23.1)$^{\$}$ (63.4%)	*P* = 0.01 *P* = 0.40 *P* = 0.02	167.7 (23.1) (39.3%)	141.1 (29.1)$^{\$}$ (63.0%)	*P* = 0.01 *P* = 0.38 *P* = 0.02
Fat intake (g day^{-1}) (PTC %)	59.4 (21.3) (37%)	26.7 (11.3)$^{\#}$ (22.6%)	*P* = 0.01 *P* = 0.33 *P* = 0.03	63.2 (11.3) (37.2%)	28.1 (9.3)$^{\#}$ (22.8%)	*P* = 0.02 *P* = 0.36 *P* = 0.03
Protein intake (g day^{-1}) (PTC %)	76.1 (18.1) (23.4%)	55.1 (11.3)$^{\&}$ (23.0%)	*P* = 0.03 *P* = 0.52 *P* = 0.04	75.0 (12.1) (23.5%)	61.2 (11.9)$^{\&}$ (23.2%)	*P* = 0.02 *P* = 0.43 *P* = 0.03
Fibre intake (g day^{-1})	15.1 (7.0)	18.3 (3.9)	*P* = 0.18 *P* = 0.51 *P* = 0.21	15.5 (7.2)	18.1 (5.2)	*P* = 0.21 *P* = 0.53 *P* = 0.18

Statistical differences $P < 0.05$, in each genotype group (*calorie intake, $carbohydrate intake, #fat intake, &protein intake) No statistical differences between genotype groups.
PTC, percentage of total calorie.

Table 3 Anthropometric parameters and blood pressure after dietary intervention [mean (SD)]

	n = 122					
	GG (*n* = 26)			GA + AA (*n* = 96)		
Parameters	Basal	3 months	*P* Time Genotype Genotype × time	Basal	3 months	*P* Time Genotype Genotype × time
BMI	39.3 (2.1)	36.7 (2.0)*	*P* = 0.01 *P* = 0.40 *P* = 0.03	39.5 (2.0)	36.9 (2.2)*	*P* = 0.005 *P* = 0.30 *P* = 0.01
Weight (kg)	101.1 (8.5)	93.3 (7.1)$	*P* = 0.02 *P* = 0.45 *P* = 0.02	101.9 (7.1)	93.6 (8.1)$	*P* = 0.003 *P* = 0.25 *P* = 0.01
Fat mass (kg)	45.4 (5.1)	40.2 (6.0)#	*P* = 0.02 *P* = 0.51 *P* = 0.03	45.7 (5.0)	40.4 (5.1)#	*P* = 0.03 *P* = 0.43 *P* = 0.02
WC (cm)	118.3 (7.1)	113.2 (5.0)&	*P* = 0.01 *P* = 0.50 *P* = 0.02	119.2 (6.9)	114.0 (5.1)&	*P* = 0.01 *P* = 0.48 *P* = 0.02
SBP (mmHg)	135.1 (7.0)	122.3 (5.2)**	*P* = 0.001 *P* = 0.21 *P* = 0.01	135.8 (6.2)	122.9 (5.2)**	*P* = 0.001 *P* = 0.18 *P* = 0.01
DBP (mmHg)	80.8 (5.1)	77.3 (3.2)***	*P* = 0.01 *P* = 0.41 *P* = 0.02	80.3 (4.0)	77.2 (5.0)***	*P* = 0.04 *P* = 0.60 *P* = 0.03

Statistical differences $P < 0.05$, in each genotype group (*BMI, $weight, #fat mass, &WC, **SBP, ***DBP) No statistical differences between genotype groups.
BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; WC, waist circumference.

antipsychotic drugs, as well as for differing durations. Finally, the endpoints for weight loss were different in the studies.

As far as we are aware, there are no studies available in the literature evaluating the effect of this polymorphism on the secondary metabolic effects of weight loss after dietary intervention. In one study, Crescenti *et al.* [20] evaluated the effect of soluble fibre (Plantago Ovata husk) versus placebo according to some SNPs, with one of them being rs7799039. After 8 weeks of treatment, Plantago ovata consumption lowered the plasma cholesterol concentrations to a greater extent in non-A allele carriers compared to non-A allele carriers. In another prospective cohort of pregnant females, the AA genotype reported a higher energy intake (higher fat) and a higher increase in triglyceride concentrations during pregnancy than females without the A allele [21]. In our study, non-A allele carriers have a greater decrease in LDL-cholesterol and triglyceride levels than non-A allele carriers despite having the same significant weight loss. Our results suggest that this genetic variant of the *LEP* gene may induce an alteration in the leptin-mediated signalling pathway and impair the peripheral effects of leptin on lipid metabolism. Perhaps a direct effect of leptin on lipid oxidation could also be implied [22]. However, a recent study in pregnant women [23] showed how this SNP can affect weight gain during pregnancy, although it did not modify the concentration of circulating leptin. Therefore, an indirect effect through leptin does not appear to be possible.

All of these controversial findings are not well elucidated in the literature. The different findings may possibly be a result of diversity in the genetic background of the populations, or interactions with other pathways of leptin or leptin receptors [24], in addition to there being a possible influence of this risk allele on the presence of metabolic syndrome [25] or even cardiovascular events such as heart attack [26].

The present study has several limitations. First, the inclusion in the trial of obese subjects without established cardiovascular disease does not allow generalisation of the results beyond a population of obese individuals without comorbidities. Second, we only analysed one SNP of the *LEP* gene, and so other genetic variants could be related to the findings. Third, many uncontrolled factors could influence our results (epigenetic, hormonal status and level of physical activity). Fourth, the absence of a leptin determination might be a bias. Finally, the self-reported dietary intake is not reliable and might include bias of under- or over-reporting.

Table 4 Change of biochemical parameters after dietary intervention [mean (SD)]

	n = 122					
	GG (*n* = 26)			CA + AA (*n* = 96)		
Biochemical parameters	Basal	3 months	*P* Time Genotype Genotype × time	Basal	3 months	
Glucose (mg dL^{-1})	102.3 (6.1)	98.6 (5.1	*P* = 0.18 *P* = 0.53 *P* = 0.14	100.1 (7.1)	97.9 (7.0)	*P* = 0.12 *P* = 0.51 *P* = 0.21
Total cholesterol (mg dL^{-1})	217.1 (12.7)	192.1 (8.2)*	*P* = 0.01 *P* = 0.23 *P* = 0.04	216.9 (5.1)	208.8 (9.2)	*P* = 0.43 *P* = 0.51 *P* = 0.19
LDL-cholesterol (mg dL^{-1})	138.1 (4.3)	117.4 + 6.1)$	*P* = 0.02 *P* = 0.33 *P* = 0.01	134.6 (8.1)	129.2 (9.9)	*P* = 0.60 *P* = 0.81 *P* = 0.32
HDL-cholesterol (mg dL^{-1})	57.1 (4.1)	56.9 (5.2)#	*P* = 0.45 *P* = 0.71 *P* = 0.69	57.2 (5.1)	57.8 (5.4)	*P* = 0.31 *P* = 0.63 *P* = 0.34
Triglycerides (mg dL^{-1})	110.4 (12.1)	95.1 (11.2)##	*P* = 0.01 *P* = 0.24 *P* = 0.02	112.1 (13.2)	109.8 (9.1)	*P* = 0.10 *P* = 0.20 *P* = 0.12
Insulin (mUI L^{-1})	15.7 (1.9)	12.6 (1.1)&	*P* = 0.01 *P* = 0.24 *P* = 0.02	16.1 (1.1)	12.9 (1.9)&	*P* = 0.001 *P* = 0.12 *P* = 0.003
HOMA-IR	4.1 (0.3)	3.3 (0.2**	*P* = 0.03 *P* = 0.23 *P* = 0.04	4.2 (0.2)	3.3 (0.4)**	*P* = 0.01 *P* = 0.31 *P* = 0.02

Statistical differences $P < 0.05$, in each genotype group (*total cholesterol, $low-density lipoprotein-cholesterol, #high-density lipoprotein-cholesterol, ##triglycerides, &insulin, **HOMA-IR). No statistical differences between genotype groups.
HOMA-IR, homeostasis model assessment.

In summary, subjects with the A allele of the rs7799039 variant in the *LEPR* gene showed a significant improvement LDL-cholesterol and triglycerides levels after weight loss secondary to a pMR hypocaloric diet.

Conflict of interests, source of funding and authorship

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DAL and JJL designed the study an wrote the article. OI and JJL performed the nutritional evaluation. DP and DL performed the biochemical evaluation. All authors critically reviewed the manuscript and approved the final version submitted for publication.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with guidelines (delete as appropriate). The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned (please add in the details of any organisation that the trial or protocol has been registered with and the registration identifiers) have been explained.

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