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COVID-19

A UK survey of nutritional care pathways for patients with COVID-19 prior to and post-hospital stay

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Abstract

Background: During the global COVID-19 pandemic, UK dietitians have delivered the best care to help patients recover from the infection. The present study examined the development and evaluation of care pathways to manage nutritional care of patients following COVID-19 infection prior to and after discharge.

Methods: Registered UK dietitians completed an online questionnaire comprising 26 questions about the development of a pathway, its use, evaluation and training needs.

Results: Of 57 responses from organisations, 37 (65%) were involved in the planning/management of nutritional care. Only 19 responses had a new or adapted COVID-19 pathway. Of these, 74% reported involvement of dietetic services, 47% reported > 1 eligibility criteria for pathway inclusion and 53% accepted all positive or suspected cases. All respondents used nutritional screening, first-line dietary advice (food first) and referral for further advice and monitoring. Weight and food intake were the most used outcome measure. All pathways addressed symptoms related to nutrition, with the most common being weight loss with poor appetite, not being hungry and skipping meals in 84% of pathways. Over half of respondents (54%) planned to evaluate their pathway and 83% reported that they were 'very or reasonably confident' in their team's nutritional management of COVID-19. Less than half (42%) reported on training needs.

Conclusions: Despite challenges encountered, pathways were developed and implemented. Dietitians had adapted to new ways of working to manage nutritional care in patients prior to and after discharge from hospital following COVID-19 infection. Further work is needed to develop strategies for evaluation of their impact.

KEYWORDS

COVID-19 infection, nutrition, care pathway, dietitians, nutritional care

INTRODUCTION

Nutrition is a crucial part of the recovery process for all patients with COVID-19, particularly for those who have experienced cardiac or pulmonary complications, as well as

for cases where frailty, sarcopenia and malnutrition have developed or been exacerbated.¹ By August 2020, more than 95 000 patients with COVID-19 infection had been cared for in hospitals across England alone¹ and, although the majority of patients may have recovered from the acute phase and been discharged from hospital, the focus has turned towards

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their recovery as the longer-term effects of the virus and its treatment become evident.

COVID-19 infection presents with a diverse range of symptoms that may adversely impact on nutritional status in patients. These include changes in taste and smell, loss of appetite and gastrointestinal symptoms such as diarrhoea and vomiting.² This poses new challenges for the nutritional care of patients who have experienced COVID-19 infection. National Health Service (NHS) England recognises the role of the dietitian in ensuring adequate nutrition and hydration to prevent malnutrition in patients following hospital discharge in their report 'After-Care Needs of Inpatients Recovering from COVID-19'. In May 2020, the British Dietetic Association (BDA) published the 'Nutrition and the COVID-19 Discharge Pathway'³ emphasising the importance of screening for malnutrition in patients with COVID-19 infection. It also called for policy makers, as well as healthcare and dietetic leaders, to take action to ensure that patients have access to appropriate nutrition, with expert guidance from dietitians as part of multidisciplinary rehabilitation pathways. The European Society for Parenteral and Enteral Nutrition (ESPEN) has also produced clinical guidance to inform healthcare rehabilitation pathways to ensure that nutrition is considered at every stage of the patient's journey.⁴ As information about COVID-19 infection accumulates, there remains a need to develop the evidence to inform new rehabilitation pathways and thus optimise recovery and reduce the likelihood of further deterioration. Care pathways have been used in the NHS from the mid to late 1990s onwards and are regarded as mechanisms for ensuring patient safety, equity in the quality of treatment, optimal use of resources, and a way to improve the efficiency and effectiveness of the care process by integration. They are designed to be a helpful tool for routing patients through the system and are regarded as patient-centred by allowing individualisation. They plot out the optimal course of treatment for an illness with prompts for relevant interventions by different professionals, and as such are ideally multidisciplinary.⁵ Flexibility and adaptability are paramount.⁶ Therefore, as an initial step towards identifying best practice to inform new care pathways, we report the findings from a national survey. The survey aimed to provide new information about nutritional care pathways to help manage patients with COVID-19 prior to and following discharge from hospitals. The key research questions were:

- What nutritional care pathways have been implemented by dietitians or their organisations to manage patients with COVID-19 infection prior to and post-discharge?
 - Have these pathways of care been adapted from other established pathways, or are new pathways being developed?
 - Which patients are being targeted by the pathway and why?
 - Which elements are included in the pathway of care and how are they measured?
 - Are pathways being evaluated and how?
- What are dietitians' views on the efficacy of the pathways and how confident are they in managing the nutritional consequences of COVID-19 infection?

METHODS

The present study employed a cross-sectional, anonymous online survey of UK dietitians. Ethical approval was obtained from Bournemouth University's Research Ethics Committee (ID 32676).

Questionnaire development

A questionnaire consisting of 26 questions was developed for the study by the project team. The questionnaire was divided into six main sections: (i) eligibility and respondent details; (ii) pathways related to the nutritional management of patients with COVID-19 infection; (iii) assessment of nutritional status and specific symptoms that could influence nutritional status; (iv) advice provided; (v) outcome measures used; and (vi) plans for evaluation and training needs. The survey questions included a combination of open and closed questions with categorical responses and Likert scales to rank responses about perception of using the pathway and confidence in the nutritional management of patients with COVID-19 (see Supporting information, Doc. S1).

Face and content validity were established by piloting the questionnaire with subject experts ($n = 6$) and clinical dietitians ($n = 6$). Subject experts assessed the content validity of the questionnaire and nominated clinically practicing dietitians to assess face validity to ensure clarity, readability and comprehension, as well as time taken to complete the questionnaire. All dietitians were based in England and worked in a combination of settings, including three from community, two from hospital, and one from hospital and community settings. Three of the six dietitians specialised in the care of older adults, two in community services and one in respiratory medicine. Amendments to the survey highlighted during the piloting phase were made prior to national distribution. The survey could be completed within approximately 15–20 min. The online survey JISC Online Surveys^{®7} was used for distribution.

Sampling and recruitment

A convenience sample of UK Health and Care Professions Council registered dietitians and active members of the BDA formed the sampling frame. Inclusion criteria were dietitians involved in the planning and/or management of the nutritional care of patients with COVID-19 infection at their Trust or Health Board. Exclusion criteria comprised non-practising dietitians, retired dietitians, paediatric dietitians, exclusively academic dietitians, student dietitians and dietitians practising outside of the UK. Dietitians were invited to complete the survey via an e-mail by the BDA and a survey

link shared via social media platforms and direct email to BDA Special Interest Groups. Reminders were shared via social media platforms three times per week during the time that the survey remained open. Only one response per organisation was required and therefore participants were asked to complete the survey and to discuss with colleagues on behalf of their organisation. A PDF version of the survey was made available to download so that a collaborative response could be achieved per pathway by an organisation. Information for potential participants was provided on the front page of the survey and respondents were asked to acknowledge they had read this information before completing the survey. Consent was presumed through participation in the survey and all responses were anonymous. The survey was open between 22 June 2020 and 12 July 2020, approximately 3 months after the COVID-19 outbreak in the UK.

Statistical analysis

Descriptive statistics are reported such as frequencies for the categorical data using Excel for Office 365 (Microsoft Corp.). Free text responses were listed verbatim then categorised by the research team using qualitative content analysis.⁸

RESULTS

In total, 57 responses were received. Of these, 37 (65%) respondents were involved in the planning and/or nutritional management of patients with COVID-19 infection (Figure 1). There were 19 respondents who had a new or adapted COVID-19 pathway for the nutritional care of patients with COVID-19 infection. The main characteristics are shown in Table 1.

Pathways related to patients with COVID-19

The different approaches to developing a pathway of care for patients recovering from COVID-19 infection are shown in Table 2. Eleven (30%) of the 37 respondents reported developing a new or adapting an existing dietetic pathway and eight (22%) had developed a new or adapted an existing multidisciplinary pathway. Eight (22%) of 37 respondents were in the process of developing a pathway, six (16%) had made no changes and four (11%) wanted to implement a pathway.

Of the 19 participants who had developed a new or adapted a pathway, 10 (53%) respondents included patients who were COVID-19 positive or who had suspected infection and nine respondents (47%) reported a range of more than one eligibility criteria for inclusion onto their pathway (Table 3).

The content of pathways varied, although all pathways included nutritional screening, first-line nutrition advice and referral for further nutrition advice and monitoring (Figure 2). Most pathways included nutritional assessment

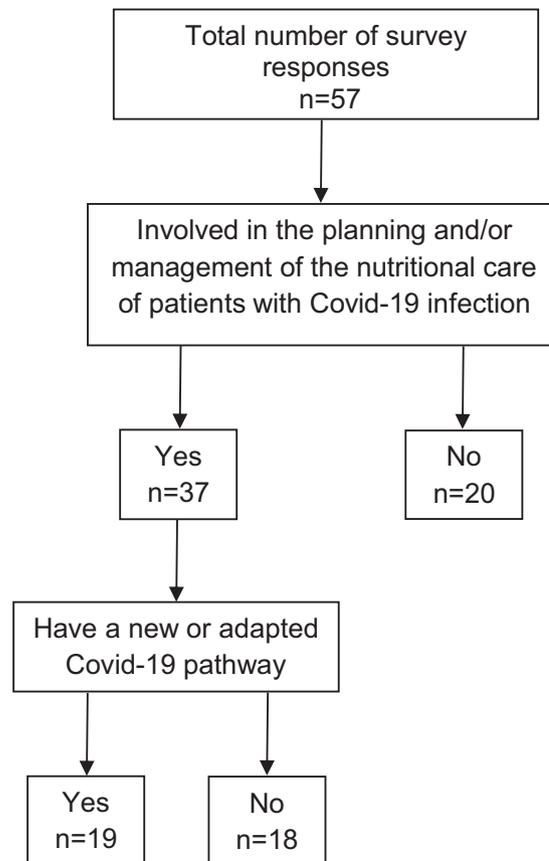


FIGURE 1 Number of respondents involved in planning and/or management of nutritional care and have a COVID-19 pathway for patients with COVID-19 infection

and oral nutritional supplements (ONS) as part of first-line intervention, activity or exercise advice. Less than half assessed COVID-19 infection-specific symptoms, or patients referred to other professionals or to social care.

Nutritional screening and assessment

Table 4 shows the nutritional screening and assessment tools reported in the pathways. The majority used the Malnutrition Universal Screening Tool ('MUST')⁹ and almost all used the ABCDE (Anthropometric, Biochemical, Clinical, Dietary, Environmental) process for nutrition assessment.

First-line advice

All respondents reported using written or online food first information and the use of locally developed resources. Other resources reported were those available from the BDA, Nutrition and Diet Resources UK, Malnutrition Pathway COVID-19 and Malnutrition Task Force/Age UK (<https://www.malnutritiontaskforce.org.uk/coronavirus-information-hub>). A variety of ONS were prescribed (see Supporting information, Doc. S2).

TABLE 1 Summary showing the characteristics of the survey respondents involved in the nutritional management of patients with COVID-19 infection ($n = 37$)

	<i>n</i>	%
Country		
England	27	73
Scotland	7	19
Wales	2	5
Northern Ireland	1	3
Clinical setting		
Hospital	13	35
Community	11	30
Hospital and community	11	30
Mental health	1	2.5
Medicines optimisation	1	2.5
Speciality		
Community care	13	35
Critical care	5	14
Other ^a	5	14
Non-clinical management	4	11
Care of older adults	4	11
General medicine	3	8
Multiple specialities	3	8

^aPaediatrics, oncology, mental health, rehabilitation, catering.

Monitoring of specified outcomes

All 19 respondents reported on the outcome measures monitored routinely in the pathway. The outcome measures monitored depended largely on the setting (e.g., critical care, general ward or community). Weight was monitored as an outcome in 17 (89%) pathways and food intake was monitored in 14 (74%) pathways. Of these, nine (64%) respondents used diet charts or tables and seven (50%) used dietary recall. Patient-specified goals were measured in 50% of all pathways. Activities of daily living were monitored in six (33%) pathways, physical function in five (28%) pathways and handgrip strength in two (11%) pathways. Two (11%) respondents noted the difficulty in recording outcome measures as a result of virtual clinics. Three respondents reported using mid upper arm circumference (MUAC). However, other outcomes commonly undertaken by dietitians including MUAC were measured by other healthcare professionals such as nursing staff because of restricted access to the wards for dietitians.

Assessment of COVID-19-specific symptoms

Nineteen respondents reported on the assessment of COVID-19 symptoms related to nutrition (Figure 3). A variety of symptoms were assessed in the majority of pathways, including not hungry at mealtimes and/or skipping

meals (84%), poor appetite (84%) and taste changes (79%). Symptoms less likely to be assessed were indigestion or heartburn (32%), bloating (37%) and chewing problems (37%).

Figure 4 shows that the most frequently assessed physical or functional symptoms were weight loss (90%), energy levels (74%), weakness (74%), shortness of breath (74%) and muscle loss (68%). Other symptoms such as pain and feeling drowsy or sleepy or fatigued were less likely to be assessed as part of the pathway. The most frequently assessed emotional or psychological symptoms were low mood (63%), anxiety (42%) or sleep disorders (32%) (Figure 5).

Regarding the provision of advice or resources with respect to management of COVID-19-specific symptoms, 12 (63%) respondents reported this for eating and drinking with breathlessness, 10 (53%) respondents reported this for managing loss of taste and smell, 10 (53%) respondents reported this for managing a dry mouth, 10 (53%) respondents reported this for prescription of ONS, seven (37%) respondents reported this for advising on purchasing nutritional supplement drinks, six (31%) respondents reported this for managing diarrhoea or other gastrointestinal disturbances and one (5%) respondent reported this for recommending multivitamin and mineral supplements.

Evaluation of the pathway

Ten (54%) of the respondents planned to evaluate the pathway. Approaches reported for evaluating the pathways comprised monitoring and review via colleagues about the discharge and review process; patient satisfaction in clinics; staff feedback based on qualitative and quantitative feedback; patient reported outcomes and retrospective audit of pathways based on key performance indicators, such as number of referrals, patients reviewed in virtual clinics, patient symptoms, nutrition support interventions used and assessment of patient perceptions of virtual clinics.

Confidence in using the pathway

Eight (42%) respondents reported that the pathway was working 'reasonably well' and nine (47%) preferred not to comment on the effectiveness of the pathway as a result of the short length of time that it had been in place. Two (11%) respondents, who had created a new multidisciplinary pathway, reported that these pathways were not working well. The first was based in critical care and a lack of resources for the large number of patients was suggested as the main reason why the pathway was not working well. The second pathway was involved in the management of community services, and issues surrounding remote working, such as educating staff and raise awareness of the pathway, were considered to be barriers to the success of their pathway.

TABLE 2 Pathway approaches reported and professional involvement in developing or adapting a pathway (*n* = 19 respondents)

Yes – it is a new dietetic-specific pathway developed specifically for COVID-19 patients	Yes – it is an adapted dietetic-specific pathway of care (e.g., frailty, pulmonary, general rehabilitation, etc.)	Yes – it is a new MDT pathway developed specifically for COVID-19 patients	Yes – it is an adapted MDT pathway of care (e.g., frailty, pulmonary, general rehabilitation, etc.)
Dietetics department	Advanced specialist dietitians from ITU and medical teams	Dietetics, physical health lead nurse	Clinical service lead therapist surgery, trauma and orthopaedics, cancer, respiratory
Community and acute clinical leads	Nutrition and dietetics	Adult dietetics	Adapted post critical care rehabilitation pathway
Dietetic clinical leads for acute, nutritional support and mental health		Physiotherapists, occupational therapists, dietitians, speech and language therapists and social services – new respiratory rehabilitation pathway	Physiotherapists, speech and language therapists and dietitians – under development
Hospital dietitians Prescribing support dietitian CCG		Respiratory rehabilitation pathway	
Hospital nutrition and dietetic services Community nutrition and dietetic services		New AHP Integrated rehabilitation pathway – dietetics, psychology, physiotherapists, speech and language therapists, occupational therapists and podiatry	
Adapted dietetic pathway			
Dietitian and input for MDT pathway for post COVID patients			
Acute dietetic services			
Clinical dietetic leads in acute and community settings			
9	2	5	3

Abbreviations: CCG, clinical commissioning group; ITU, intensive therapy unit; MDT, multidisciplinary team.

TABLE 3 Criteria for inclusion in the COVID-19 pathways of care from nine respondents

Inclusion criteria ^a	
‘MUST’ score of 2 or more	3
Subjective methods (deemed to be at risk of malnutrition e.g., poor oral intake or reduced appetite)	3
Enteral tube feeding	2
Patient consistently scoring 1 or above on MUST (minimum one month between screening or bi-weekly as appropriate)	1
Length of stay in ICU of 4 or more days	1
Patients with COVID-19 infection seen by dietitian prioritising those in ICU and those who required oxygen on ward	1
Dysphagia and/or strictures requiring texture modification, assessed by a Speech and Language Therapist	1
On an Oral Nutrition Supplement prescription (regardless of weight/ weight loss)	1
BMI < 18.5 kg m ⁻²	1
BMI < 205 kg m ⁻² with unintentional weight loss of > 5% over the past 3–6 months	1
Patient of any BMI who presents with ≥ 10% unintended weight loss over the previous 3–6 months	1
All admitted in-patients with COVID-19	1

Abbreviations: BMI, body mass index; ICU, intensive care unit; MUST, Malnutrition Universal Screening Tool.

^aMore than one criteria was reported.

Main difficulties setting up or adapting pathway

Of the 19 respondents who developed or adapted a pathway, 18 reported the difficulties they faced setting up

or adapting the pathway (Table 5). These difficulties included issues related to remote working, work pressures, redeployment of dietitians to other roles not related to nutritional care and reduced access to wards for dietitians

FIGURE 2 Aspects of nutritional care included in new or adapted nutritional care pathways for patients following COVID-19 infection ($n = 19$). ONS, oral nutritional supplement

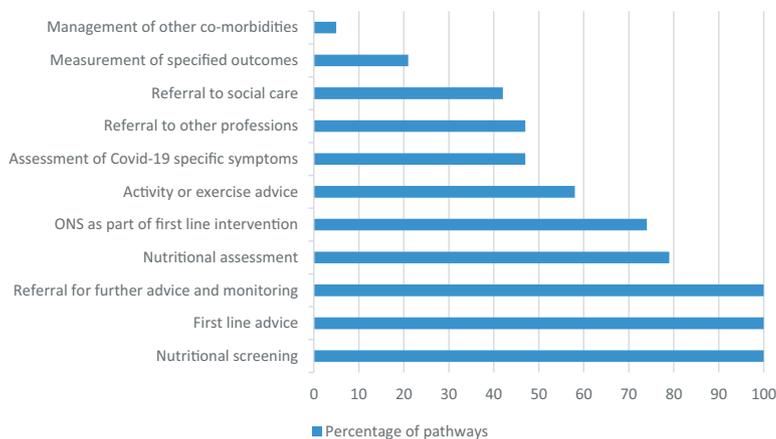


TABLE 4 Nutritional screening and assessment tools used for the nutritional care pathways for patients with COVID-19 infection ($n = 19$)

	<i>n</i>	%
Nutritional screening		
'MUST'	14	73
Patients Association Nutrition Checklist	2	11
Combination of 'MUST' and local tool	1	5
Local tool	1	5
WASSP ^a	1	5
Nutritional assessment		
ABCDE ^b	17	90
Patients Association Nutrition Checklist	1	5
Electronic patient records	1	5

Abbreviation: MUST, Malnutrition Universal Screening Tool.

^aWeight, Appetite, Ability to eat, Stress factors, Pressure sores/wounds.

^bAnthropometric, biochemical, clinical, dietary, environmental.

(possibly as a result of limits on personal protective equipment).

Remote working issues included difficulties communicating with other teams to decide outcome measures, IT difficulties in setting up virtual clinics and the inability to see patients face-to-face. Work pressures were linked to low staff and high patient numbers, resulting in other priorities (such as medical emergencies) taking precedence over adherence to the pathway. The redeployment of staff and reduced access to acute wards for dietitians meant that there was less ability to see patients face-to-face, there were less dietitians to meet the need for nutritional assessment and providing training, and completing referrals was time consuming. A further challenge faced was creating a pathway that could be standardised across all specialities and multidisciplinary teams. Respondents indicated that their role and responsibilities was unclear and more clarification was needed on the advice and actions taken by all members of the multidisciplinary team involved within the pathway.

Six respondents reported how they overcame difficulties. This included reiterating the existing systems to lead nurses,

including updates (written, verbal, online) on nutrition, food and fluid guidance, and offering telephone support to the wards. One respondent indicated that they 'streamlined' patients into appropriate clinics for timely nutrition support and another reported prioritising patients and working more closely with physiotherapy.

Confidence in the nutritional management of patients with COVID-19 infection

All 37 respondents answered the question about confidence. Seven (19%) reported that they were 'very confident' in their nutritional management of patients with COVID-19, 24 (65%) were 'reasonably confident' and six (6%) were 'neutral'.

Training needs

Twenty-four respondents reported on training needs. Five (21%) wanted further training on referral processing to and within community settings to ensure patients receive required support, especially with remote delivery of pathways. Five (21%) wanted training on the long-term complications of COVID-19 and how best to support complex patients, such as those with dysphagia or gastrointestinal cancers.

Three (13%) respondents reported that continued research is required and two (8%) reported that mandatory training and the upskilling of non-acute staff to an intensive care unit settings was required. Five (21%) respondents indicated that training should take a multidisciplinary approach and that nutrition training and awareness should be available to non-nutrition staff.

Six (25%) respondents reported that they had found the BAPEN and BDA resources useful and that both organisations could be approached regarding the potential use of their resources as the basis of future training programmes. Six (25%) respondents suggested the type of training that they would like included online resources and webinars to support their learning needs.

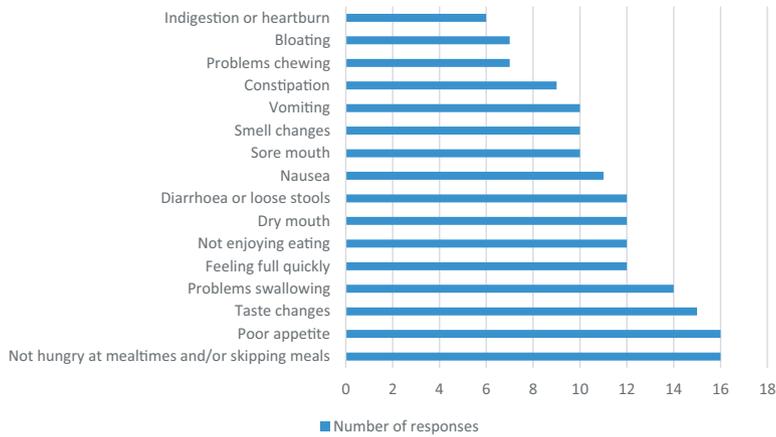


FIGURE 3 Assessment of nutrition-related symptoms included in nutritional care pathways for patients following COVID-19 infection ($n = 19$)

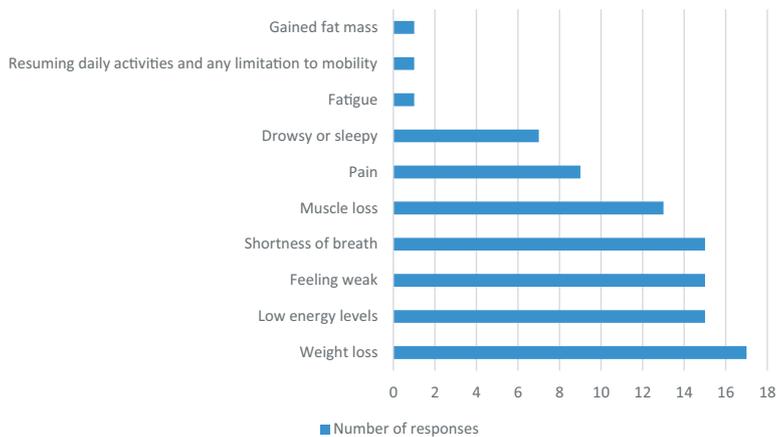


FIGURE 4 Assessment of physical or functional symptoms associated with COVID-19 infection ($n = 19$)

DISCUSSION

The present study is the first of its kind to examine nutritional care pathways for patients prior to and following discharge from UK hospitals following COVID-19 infection. We have provided new information on what care pathways (multidisciplinary and dietetic specific) had been implemented or were planned for development indicating the urgent need for new pathways or the adaption of current pathways. The major findings were significant inconsistencies in the development and content of the pathways and, at this stage, the evaluation of impact of the pathways did not appear to be prioritised or planned. Neither of these findings are surprising given the nature of the global pandemic and the need to respond rapidly to an urgent and critical situation. Nevertheless, COVID-19 infection will remain prevalent in the community and so it is worth revisiting the pathways initiated speedily to review, evaluate and modify them with respect to incorporating acquired knowledge and experience.

The survey showed no consistent approach to pathway development; there were both dietetic-specific and multiprofessional pathways developed. Most were newly developed rather than comprising the adaptation of current pathways, which might suggest that the extreme situation could not

be successfully mapped onto other existing pathways, or that other pathways simply did not exist. Nevertheless, a minority of respondents did adapt existing pathways, such that, in some areas, this was clearly a possibility or seen as the most efficient approach. Because a care pathway for COVID-19 infection should map the patient's journey to recovery, it is inevitable that it will cross care settings and involve a variety of professional groups. Thus, it might be more effective to take a multidisciplinary approach, although the critical situation that the pandemic presented may have resulted in a more pragmatic approach, with a care pathway for a single discipline being easier to develop than one involving many professions. Further work needs to explore the contribution of dietitians and the different professions involved in the development or adaptation of pathways and their responsibilities.

The survey attempted to examine the elements of the pathways in some detail. There was a lack of consistency in the criteria for inclusion of a patient on the pathways, where criteria beyond a positive or suspected COVID-19 infection were used. Criteria included both subjective (poor appetite or intake, etc.) and objective measures ('MUST' score, low body mass index, intensive care unit admission for a specified period). Criteria are useful to target treatments to patients most in need and, in these

FIGURE 5 Assessment of emotional or psychological related symptoms associated with COVID-19 infection ($n = 19$)

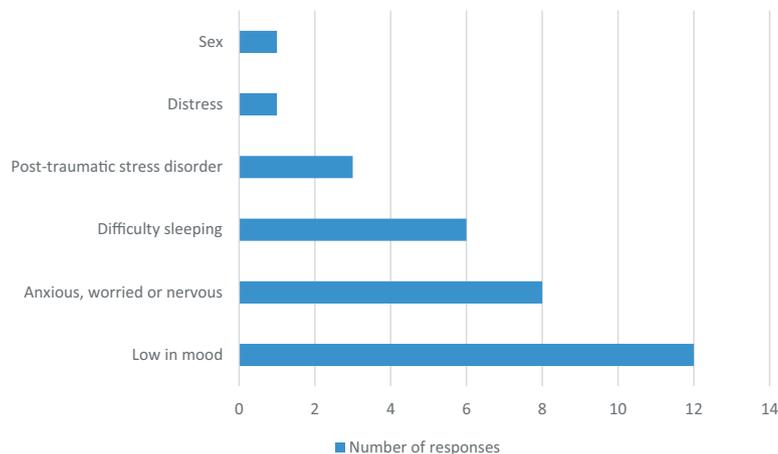


TABLE 5 Main difficulties associated with setting up or adapting a nutritional care pathway for patients with COVID-19 infection from 18 respondents

Difficulty ^a	<i>n</i>	%
Remote working including setting up virtual clinics	4	22
High volume of patients and lack of staff/redeployment of staff	3	17
Time constraints for planning and training staff	3	17
Working with other specialities to standardise pathways and provide support and clarification on what advice can be provided	3	17
Long-term planning – funding and management	3	17
Missed referrals	2	11
Staff adherence to the pathway	2	11
Agreeing outcome measures	1	6
Other issues not related to COVID-19	1	6

^aMore than one difficulty was reported.

care pathways, the prevention of malnutrition was the key outcome. Thus, criteria to select those most at risk of subsequently developing malnutrition were used. Because the complications and outcomes of COVID-19 infection were largely unknown at the time that these pathways were developed, many respondents had decided that all infected patients should be seen regardless of other risk factors. However, differences observed in the criteria for inclusion of patients onto the pathways indicates a lack of clarity about which patients will benefit most. Therefore, there is a need for more research aiming to understand the consequences of COVID-19 infection better and to standardise the approaches used nationally.

Consistent features of all of the pathways were nutrition screening, use of first-line dietary advice, and referral for further nutrition support and monitoring. Because these are important and basic steps in the assessment and treatment of people at risk of malnutrition,¹⁰ it is unsurprising these were used in all pathways. Furthermore, evidence suggests that nutritional support can prevent or help to reverse the problems associated with undernutrition.¹¹ The most commonly used screening tool was ‘MUST’ and, because this is the most widely used tool in the UK,¹² this is to be expected. In addition, screening and nutritional support are the first two statements in the ESPEN guidance for nutritional

management of COVID-19 infection, a practical document published early in the pandemic.⁴

Similarly, more detailed nutritional assessment, as well as ONS as part of the first-line intervention, were used in most pathways. Trials using ONS during acute illness have shown reduced length of hospital stay¹³ and reduced re-admission.¹⁴ Additionally, in respiratory disease (chronic obstructive pulmonary disease), ONS have been shown to improve peripheral muscle strength¹⁵; thus, there is good evidence for this element in these pathways. However, compliance with ONS prescriptions is variable¹⁶ and so on-going dietetic support may be needed to optimise consumption.

Interestingly, more than half the nutritional care pathways specified including advice on activity or exercise. Muscle mass is crucial for health and independence¹⁷ and it is lost during ageing.¹⁸ The best evidence for retention of muscle mass and regain after loss is resistance exercise.¹⁹ Thus, there is good evidence that exercise should be a part of optimal recovery for patients who have had reduced mobility, as well as reduced nutritional intake.

Less than half the pathways specifically assessed COVID-19-related symptoms, referred to other professions or social care, measured specified outcomes, or attempted to manage other co-morbidities. This may reflect the novel and urgent nature of the situation resulting in a need for a rapid

development of pathways and a lack of specific information about COVID-19 symptoms. However, given reports about the negative impact of COVID-19 on food insecurity,²⁰ it is important that integrated care pathways with social care are developed as part of post-discharge nutritional care. Taste changes, one of the key symptoms of COVID-19 infection,²¹ was specifically included in most pathways.

The most commonly monitored outcomes in the pathways were body weight and food intake. These are important and obvious indicators of the response to nutritional treatment, or further deterioration in condition and a rising risk of malnutrition. We did not investigate how body weight was measured or self-reported. However, the data indicate that other tools were used to identify risk of malnutrition, such as the Patients Association Nutrition Checklist, which does not require a measure of body weight. Although a recent study shows the potential for e-scales in clinical practice,²² further studies need to explore the validity of collecting measures of body weight virtually or the use of a validated screening tool that does not include measures of body weight. Far fewer pathways measured functional measures, such as hand-grip strength and activities of daily living, and others took the approach of using achievement of patient specified goals. There appeared to be considerable problems in obtaining outcome measures, particularly objective measures, as a result of virtual clinics and other infection control measures.

At the time of the survey, 54% of respondents who had developed or adapted a pathway were in the early stages of planning to evaluate their pathways. None reported a plan for evaluating the impact of the pathway on patients. The evaluation of any new service development is important in terms of checking that it is working effectively and achieving the desired goals.²³ These data indicate that many dietitians find it challenging to design and implement suitable evaluation plans. Some respondents reported that they felt the pathway was working reasonably well, whereas others did not, highlighting the need for on-going evaluation. The challenges of setting up or adapting the pathways were highlighted and reflect the unprecedented situation that the pandemic generated. Some of the difficulties, such as time constraints and working across disciplines, are not specific to the pandemic but were potentially compounded and intensified in the pressurised working environment that existed. The dietitians involved also demonstrated their problem-solving capabilities by engaging practical and often simple solutions to the challenges they faced. A frequent drawback of care pathways is that they are less patient-centred and flexible and often do not embed the patient's voice in their journey through the 'pathway'.

Most respondents responded that further training about nutrition-related issues in COVID-19 infection was required. Most stated specific areas for training, with these being varied, including process issues (referral within the community), other staff training needs (nutrition training for non-nutrition staff) and their own need for more information (long-term complications and supporting complex patients). There was a recognition that as new aspects of

managing COVID-19 infection would continue to rapidly evolve, such as long COVID-19.²⁴ New approaches to online training and webinars were received favourably and were considered as an innovative approach for nutrition training that could be further developed.²⁵

We recognise the limitations that the survey may not provide a complete picture of practice from Health Trusts and Health Boards in the UK. Although most of the respondents were from England, there was representation from Scotland, Wales and Northern Ireland, as well as from a range of different clinical settings and specialities. The survey was strengthened by respondents having the opportunity to add free-text answers for most questions. The research was undertaken over a short time frame towards the end of the first wave of the COVID-19 outbreak (in June 2020), which might have limited the number of dietitians able to respond as a result of time constraints. Nevertheless, although it was too early for some organisations to provide information and some organisations were still in the process or were intending to develop new pathways, these are encouraging developments and require further investigation. Surprisingly, there were 20 respondents who were not involved with the planning and/or management of nutritional care of patients with COVID-19 infection. Further research is needed to understand whether there has been dietetic involvement in nutritional care for COVID-19 infection subsequent to the time of the present study, given that there has been a second wave of the pandemic at the end of 2020/early 2021, with greater numbers of people with COVID-19 infection being admitted to UK hospitals.

CONCLUSIONS

The present study provides new information on the development of new nutritional care pathways for patients recovering from COVID-19 infection, as well as what pathways had been implemented to date or were under development. New or adapted post-hospital discharge pathways will support the transition from hospital to home and particularly benefit those with long COVID-19. Dietitians have had to respond rapidly and have adapted to new ways of working to overcome the challenges encountered. Further work will use these findings, combined with a review of the current literature, to design an evidence-based pathway for the management of malnutrition in patients prior to and after discharge from hospital following COVID-19 infection and to enable more consistency and standardisation of practice.

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CONFLICT OF INTERESTS

The authors have no conflicts of interest.

AUTHOR CONTRIBUTIONS

All authors contributed to the conception, design and piloting of the questionnaire and interpretation of the data. VL designed, implemented and analysed the online questionnaire. VL, JM and MH drafted the manuscript. All authors critically reviewed the content of all drafts and have approved the final version of the manuscript submitted for publication.

ETHICAL APPROVAL STATEMENT

Ethical approval was obtained from Bournemouth University's Research Ethics Committee (ID 32676).

TRANSPARENCY DECLARATION

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

PEER REVIEW

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Conducting research on diet–microbiome interactions: A review of current challenges, essential methodological principles, and recommendations for best practice in study design

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Abstract

Diet is one of the strongest modulators of the gut microbiome. However, the complexity of the interactions between diet and the microbial community emphasises the need for a robust study design and continued methodological development. This review aims to summarise considerations for conducting high-quality diet–microbiome research, outline key challenges unique to the field, and provide advice for addressing these in a practical manner useful to dietitians, microbiologists, gastroenterologists and other diet–microbiome researchers. Searches of databases and references from relevant articles were conducted using the primary search terms ‘diet’, ‘diet intervention’, ‘dietary analysis’, ‘microbiome’ and ‘microbiota’, alone or in combination. Publications were considered relevant if they addressed methods for diet and/or microbiome research, or were a human study relevant to diet–microbiome interactions. Best-practice design in diet–microbiome research requires appropriate consideration of the study population and careful choice of trial design and data collection methodology. Ongoing challenges include the collection of dietary data that accurately reflects intake at a timescale relevant to microbial community structure and metabolism, measurement of nutrients in foods pertinent to microbes, improving ability to measure and understand microbial metabolic and functional properties, adequately powering studies, and the considered analysis of multivariate compositional datasets. Collaboration across the disciplines of nutrition science and microbiology is crucial for high-quality diet–microbiome research. Improvements in our understanding of the interaction between nutrient intake and microbial metabolism, as well as continued methodological innovation, will facilitate development of effective evidence-based personalised dietary treatments.

KEYWORDS

nutrition, microbiota, gastrointestinal tract

INTRODUCTION

Diet has long been recognised as a significant modulator of human health. It is now clear that the gastrointestinal (GI) microbiota is an essential mediator of this influence of diet on health. Dietary substrates are a key determinant of the

structure and function of the GI microbiome, influencing the production of metabolites and microbe–host interactions. Key observational studies^{1–3} and short-term extreme diet intervention trials^{1,4} provide examples of the nature and extent of its effect in the long- and short-term, respectively. For example, fibre deprivation alongside markedly increased

fat and protein intake has notable disruptive effects on microbial community structure, including the abundance of plant-metabolising Firmicutes.⁴ As such, the microbiome has become an attractive target for dietary interventions aiming to modulate health outcomes, with particular relevance for conditions where the microbiome is considered important in disease pathogenesis (e.g., obesity, inflammatory bowel disease and irritable bowel syndrome).^{5–7}

There are intrinsic complexities with respect to conducting studies in humans to assess diet–microbiome relationships. For example, humans are both free to select the foods that they consume and are relied upon for an accurate self-report of dietary intake. This leads to significant challenges, with under-reporting estimated to occur in 18–54% of participants in large population surveys.⁸ Individuals also consume a wide

variety of foods and, to date, food composition databases inadequately capture all dietary compounds that may have relevant effects on the microbiota. Furthermore, although the advent of high throughput sequencing technologies means that it is now possible to profile the majority of the microbiota in a rapid manner, challenges remain in linking these data to dietary intake and health outcomes, and finding consensus across studies. This may partly be a result of the significant inter-individual variation in the composition of the microbial community in the human gut, which may prime certain individuals for response/non-response to dietary interventions.⁹

To address these challenges in human diet–microbiome research, partnership across the disciplines of nutrition science and microbiology for planning, design, implementation, data analysis and interpretation is needed. The aim of this

TABLE 1 Dietary assessment methods and the key advantages and disadvantages that are relevant for diet–microbiome research

Dietary assessment method	Description	Output
Weighed food record	Participants weigh and record all food and beverages in real-time Usual duration 3–7 days	Energy, fibre, macronutrients, micronutrients, additional food constituents (depending on food composition data) Food groups Number of meals
Unweighed food record	Participants record estimated quantities of food and beverages in real-time Usual duration 3–7 days	As for weighed food record
24-h recall <i>Examples of online versions:</i> ASA24, myfood24, Intake24	Food and fluid intake between midnight to midnight day prior collected through structured interview with trained interviewer	As for weighed food record
Food frequency questionnaire <i>Examples:</i> Harvard FFQ, EPIC FFQ, AES, Food4Me	Questionnaire that assesses frequency of consumption of individual foods over a defined period (e.g., 1 year) Most include 80–120 items	Energy, fibre, macronutrients, micronutrients and additional food constituents (dependent on food composition data) Food groups
<i>Diet quality and dietary patterns</i>		
Diet quality <i>Examples:</i> HDI, HDS, HEI, AHEI, MDS	<i>A priori</i> score measuring overall healthfulness of the diet based on current evidence	Components aggregated to obtain a final score. Higher score indicates better diet quality
Diet pattern analysis	<i>A posteriori</i> approach Derives patterns using principal components/ exploratory factor analysis or cluster analysis. Patterns such as ‘prudent’ or ‘Western’ can be derived	Identifies foods consumed together (principal components analysis) or clusters individuals with differing dietary intakes (cluster analysis)

Abbreviations: AES, Australian Eating Survey; AHEI, Alternate Healthy Eating Index; ASA-24, Automated Self-administered 24-hour Dietary Assessment Tool; FFQ, food frequency questionnaire; HDI, Healthy Diet Indicator; HDS, Healthy Diet Score; HEI, Healthy Eating Index; MDS, Mediterranean Diet Score.

review is to discuss ongoing challenges in diet–microbiome research and issues for best-practice study design, as the field moves towards a goal of personalised nutrition for disease prevention, as well as therapeutic diets for a broad spectrum of microbiome-associated disorders.

Ongoing challenges in diet–microbiome research

Precision in dietary data collection

One of the most acknowledged intrinsic complexities of dietary research is that dietary intake data are measured subjectively via self-report. Provision of clear instructions

to participants prior to completion of diet recording, utilisation of resources (e.g., food models) to enhance portion size accuracy, and comprehensive cross-checking of collected data are simple yet often overlooked strategies that can improve accuracy of dietary data. With regard to energy intake, implausible data can be identified using calculations based on low and high energy intake cut-offs¹⁰ or predicted energy requirements.¹¹ The expertise of a nutrition professional for implementing these measures, as well as coding and analysing dietary data, is essential. There has also been increasing interest in capturing 24-h recall through mobile phone-based applications, some of which employ image-based technology. These tools are potentially of great value, particularly for reducing respondent burden and improving adherence and accuracy.¹² Table 1 details the major dietary

Study design	Advantages in diet–microbiome research	Disadvantages in diet–microbiome research
Experimental ‘whole diet’ interventions; supplementation studies to check background intake Cohort Case–control Cross-sectional Longitudinal	Most precise measure of actual dietary intake Useful for associating very recent dietary intake with microbiota profile Good agreement with biological dietary biomarkers	Very burdensome for participant Burdensome for researcher May influence eating behaviour May require assessment of inter-observer agreement between coders
As for weighed food record Cohort Case–control Cross-sectional Longitudinal	Less burdensome for participant than weighed food record Useful for associating very recent dietary intake with microbiota profile Low burden for participant Online versions available Multiple 24-h recalls demonstrate good agreement with dietary biomarkers Single 24-h recall acceptable for large cross-sectional studies	Burdensome for researcher May influence eating behaviour Participants may under- or over-estimate quantities Risk of recall error Requires trained interviewer Interviewer bias (data accuracy dependent on interviewer expertise, consistency between interviewers) Single 24-h recall usually not appropriate due to day-to-day variation in dietary intake
Experimental studies requiring long-term diet data Cohort Case–control Cross-sectional Longitudinal	Accounts for weekly/seasonal variation in intake Useful for assessment of habitual diet–microbiome associations Low burden for participant Simple to administer Practical for large scale studies Validated tools available for specific populations, specific nutrients	Time consuming for participant (up to 60 min) Requires mathematical skill to calculate intake using frequency categories Infrequently consumed foods may be missed due to fixed food lists Greater risk of under-reporting and error compared with other methods
Cohort Case–control Cross-sectional Longitudinal	Accounts for complexity of the diet and interactive effects of dietary factors Many indices validated by relating index score against health outcomes	Majority require nutrient intake assessment (i.e. food record, 24-h recall or FFQ) for calculating final score
Cohort Case–control Cross-sectional Longitudinal	Accounts for complexity of the diet and interactive effects of dietary factors Can be used as a covariate to determine if the effect of a nutrient is independent of the overall dietary pattern	Requires nutrient intake assessment Patterns empirically derived from data not from diet–health evidence Arbitrary decisions required (e.g., food groups, number of factors/clusters to be retained)

assessment methods and their key advantages and disadvantages in diet–microbiome research.

To overcome the limitations of self-report, biological markers can also be used as surrogate markers of recent dietary intake. Urinary nitrogen, as a marker of protein intake, is the most well validated of these biomarkers,¹³ although a variety of other metabolites have also been used to estimate food intake, including wholegrain wheat or olive oil, or nutrients, such as carbohydrate and vitamins.¹⁴ Recently, metabarcoding techniques have also been used to quantify the plant component of humans diets that is recovered in stool samples.¹⁵ Currently, these biomarker methods are limited to a finite list of dietary constituents, can be expensive, and the validity, reproducibility and sensitivity of some available biomarkers is still suboptimal.¹⁶ Furthermore, some metabolite biomarkers may also be a by-product of microbial activity, which complicates their use for estimating dietary intake. Although diet self-report will continue to be necessary for years to come, future research aiming to refine existing biomarkers and identify new biomarkers of food intake using cost-effective technologies will be important for advancing our understanding of diet–microbiome relationships.

Measuring dietary substrates available to the microbiome

Food composition data

Despite ongoing efforts to more precisely quantify dietary intake, challenges remain with regard to applying this to diet–microbiome research. First, although many dietary constituents relevant to diet–microbe interactions have been measured in foods, not all are readily quantifiable in human diets or have simply not yet been measured comprehensively across the entire food supply. For example, total fibre intake can be estimated using current methods of dietary analysis, although this is without discrimination of fibre types. This is an important shortcoming considering the vastly different physico-chemical attributes of individual fibres (including degree of polymerisation, viscosity), which influences their availability for microbial fermentation.¹⁷ Similarly, plant phenols have been shown to alter the composition of the human GI microbiota,¹⁸ although they are rarely included in food composition databases. Progress in composition analysis of these food components and development of validated tools to measure their intake will be an important step forward. Even with such advances, measurement of availability of dietary components to intestinal microbes will remain problematic. Food composition tables are based on chemical analysis of foods, which fails to account for variation in bioavailability of dietary substrates, particularly those found in plant foods.¹⁷ This, together with inter-individual variation in absorption, especially of minerals,¹⁹ limits our ability to precisely quantify the level of dietary substrates accessible to microbes.

Dietary assessment: Granular or global?

Another relevant consideration is whether a nutrient-centric or global dietary assessment should be conducted. Much of the cross-sectional work examining diet–microbiome associations in health³ or disease^{20–22} has focussed on the relationship between individual nutrients and the microbiome. This has substantially enhanced our understanding of these relationships and will continue to be relevant to measure. However, data at this granular level are increasingly recognised as failing to capture the complexity of the diet–health relationship. Dietary intake occurs in the form of food and meals, and assessing diet in this way may better explain the impact of diet on the microbiome.^{23,24} As such, a global dietary parameter, such as diet quality,^{25,26} or dietary pattern analysis,²⁷ may be an important adjunct measure. These may prove invaluable as they can be used to capture the combination and interaction of foods and nutrients consumed and assess the overall healthfulness of the diet relative to healthy eating guidelines or adherence to a specific beneficial dietary pattern such as the Mediterranean diet. Complex modelling of food intake using a tree-based alpha-diversity measure of food diversity is also possible. A recent study utilised this novel method to show that food choices are very variable across a 17-day period and that foods themselves are more strongly associated with the microbiome profile of healthy individuals than macronutrient intake, which remains relatively stable.⁹ These data further support the notion that traditional analysis of macronutrients alone may fail to uncover diet–microbiome relationships.

Relevance of microbial niches

As a result of digestion and absorption, nutrients are not universally available to microbes across the various niches of the human GI tract. In combination with variable digestive processes and motility, this leads to a unique microbiome across each GI region.^{28,29} Interpreting links between dietary intake and the GI microbial community must therefore consider substrate availability in the region sampled. However, to date, much of our understanding of diet–microbiome interactions is based on microbial profiles of stool, as a result of the relative ease of collection. Here, the luminal microbial compartment of the distal colon is reflected,³⁰ which represents the bulk of GI microbial biomass. Dietary components that are host-indigestible, namely fibre, form the key substrates for colonic fermentation, and are therefore central to microbial composition in this region. However, spill-over of excess dietary protein and fat,^{31,32} as well as the other impacts of macronutrients on host physiology, such as bile production,³³ can also influence colonic microbes, and this must be considered particularly in the interpretation of whole diet interventions in which multiple dietary changes are required. By contrast, the small intestinal microbiome is comparatively poorly characterised as a result of lower microbial densities and difficulties in sampling. However, emerging evidence suggests it also plays an important role in disease pathophysiology,^{34,35} and is involved

in immune-microbe interactions and modulating small intestinal permeability.³⁶ Although the majority of digestible dietary substrates are absorbed here, including sugars, lipids and amino acids, microbes are in competition with the host for these simple substrates, signifying the important modulatory role of diet in this region.³⁷

Microbes adherent to the mucosa, although lower in biomass, have a greater ability to interact directly with host cells compared with luminal microbes.^{38–40} Substantial evidence supports these compartments as distinct communities.^{28,40–42} Enriched with mucin-utilising organisms, the mucosal compartment, particularly in the colon, has been shown to play important regulatory roles in preserving the intestinal barrier.⁴³ Together with the small intestinal niche, the mucosal microbial community is almost never sampled in diet-microbiome studies. Emerging evidence suggests that it is influenced by host diet, including fibre availability and overall diet quality,^{44,45} and this may influence mucosal barrier function. This region may therefore be more important for the health implications of diet-microbiome interactions than previously recognised.

Understanding the microbial community structure

Methods of community assessment

The majority of diet-microbiome studies utilise amplicon sequencing of a segment of the 16S ribosomal ribonucleic acid (rRNA) gene, providing a rapid snapshot of the overall microbial community (Figure 1). Extensive, curated databases of 16S rRNA gene sequences are available that map sequences to the taxonomic assignment of organisms.^{46–48} Amplicon-based profiling studies have typically grouped sequences into clusters (operational taxonomic units, OTUs) based on a set sequence similarity. However, this reduces resolution and makes comparisons across studies difficult because each analysis will result in a unique set of OTUs.⁴⁹ More recent bioinformatics developments have led to exact determination of amplicon sequence variants (ASVs). These techniques do not rely on clustering of similar sequences, but retain all unique sequences, removing only those determined to represent sequencing error.^{49–51} This leads to improved resolution and reproducibility, with the same ASVs potentially identified across multiple different studies.

Despite these advances, the 16S rRNA gene approach has several limitations. Amplicon sequencing is based on only a short gene segment, which may not differentiate closely related organisms, and certain taxonomic groups may be under-represented depending on the hypervariable region of the gene used.^{52–54} In addition, taxonomic assignment of partial-length 16S rRNA gene sequences is often inaccurate below genus level.⁵⁵ This approach also restricts analyses to identification of bacteria and archaea, although approaches to capture fungi⁵⁶ and viruses⁵⁷ are increasing in scope. Metagenomic sequencing, and the resultant generation of more complete genomic data, is able to resolve some of the

forementioned issues, as well as provide information on the potential metabolic properties of an organism based on presence of relevant genes^{58–60} (Figure 1). However, metagenomic sequencing is more costly and analytically intensive. The use of this technique on a select number of samples in combination with 16S rRNA gene amplicon sequencing represents an alternative approach.

Reporting on taxonomy

Identifying the specific microbes that respond to dietary substrates is one of the cornerstones for understanding diet-microbe interactions and their implications for health. Sequence-based analysis of the microbiome, using ASVs/OTUs or metagenomics-based species identification, allows for very granular reporting, which is valuable given the metabolic variability of the microbial community. However, this level of detail also highlights the inter-individual variability of the microbiome from one human to the next. Reporting at higher taxonomic levels may overcome some of this variability, allowing key trends to be identified, although it will also mask potentially important detail because microbes within a taxonomic group can have different metabolic activities or differential responses to dietary substrates. Overall, there remains little consistency as to which classification or taxonomic levels are reported (e.g., ASV, genus, family and/or phylum); thus, synthesising the findings across studies remains difficult.^{61,62} Generation of publicly available raw datasets provides scope for meta-analysis of data, regardless of original analysis techniques, metrics used or taxonomic level reported. These syntheses will be invaluable for establishing further consensus in terms of specific diet-microbiome interactions.

Shortcomings of relative abundance

A key outcome in microbiome studies is microbial relative abundance, which describes the ratios of microbes that make up a given community. However, without quantification of cell numbers, it is not clear whether change in relative abundance over time is driven by a change in absolute abundance of that specific microbe, or rather collinear relationships with other members of the microbial community.^{63,64} Change in absolute abundance, as well as overall density of microbes in the GI tract, is more likely to reflect biologically relevant differences in functional outputs, such as how much of a metabolite is produced, although this is also influenced by gene/protein expression. Recently, techniques have been developed that provide quantitative data in combination with sequencing; for example, through the addition of known quantities of exogenous DNA^{65,66} or the use of flow cytometry.^{63,64}

The limitations of relative abundance are particularly important when considering responses to dietary substrates. These can take the form of 'consumption' type responses, where microbes can increase their population size when their preferred dietary derived substrate(s) are in excess. By contrast, those microbes with a 'limitation'

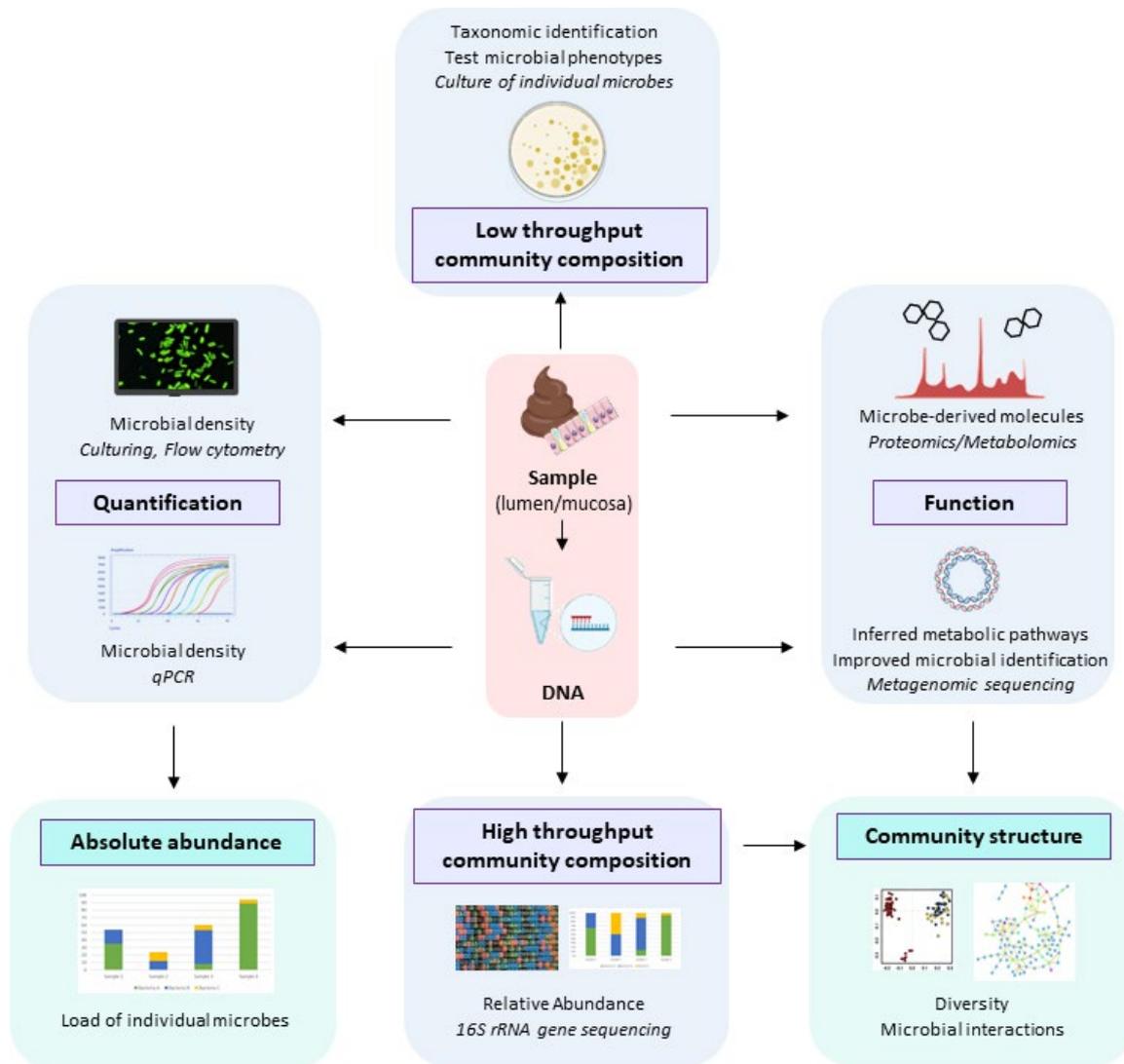


FIGURE 1 Overview of microbial analysis techniques and applications. The use of 16S ribosomal ribonucleic acid (rRNA) gene amplicon sequencing provides an overview of the microbial community and the analysis is based on relative abundance. Metagenomic sequencing, in which total genomic DNA is analysed, enables insight into potential functions encoded by microbes (e.g., substrate degradation and metabolite production). These microbial functions can be measured more directly from biological samples through techniques such as metabolomics and proteomics. Quantification of absolute abundance of microbes can be achieved directly by assessing cell numbers (culturing or flow cytometry), or through surrogate analysis of DNA, such as by a quantitative polymerase chain reaction (qPCR). Although the culture of individual microbes is low throughput, a renaissance in culture-based assessment of gastrointestinal microbes is being driven by sequence-based identification of new organisms, and is essential for fully characterising these organisms and their metabolic and functional properties

type response, are able to maintain their population when dietary substrates are limited, often as a result of the use of host-derived substances such as mucin.⁶⁷ Microbes may also appear to increase in relative abundance in certain circumstances, such as when fibre availability is low, but may actually have a stable population that becomes a proportionally larger component of the overall community because of the reduced abundances of other microbes.⁶⁸ Such collinear relationships are also complicated by microbial cooperation and cross-feeding, which is increasingly recognised as key to microbial community structure in the gut and production of health-associated metabolites such as short chain fatty acids (SCFAs).^{69–71} An enhanced understanding of these relationships will be

required for optimising intervention strategies that target the microbiome.⁶⁹

Perspectives on diversity

Diversity within the microbial community of an individual (alpha-diversity) is a frequent outcome measure in diet-microbiome studies. Individuals following a traditional agrarian-style diet higher in microbiota-accessible carbohydrates harbour higher microbial diversity than those consuming a Western-style diet.^{72–74} Despite this, short-term fibre supplementation alone does not increase diversity,⁶¹ suggesting that habitual diet may be a more important determinant. Lower diversity is frequently associated with disease states, such as Crohn's disease and obesity, and, although

meta-analyses may not always support this concept,^{75,76} diversity is an endpoint in many diet intervention studies. Although greater microbial diversity is often considered an indicator of GI health, greater diversity may not equate to increased abundances of microbes that perform beneficial functions,⁷⁷ nor is it an absolute requirement for stability and resilience of the microbial ecosystem.⁷⁸ Therefore, although alpha-diversity is an indicator of microbiota structure, it is most useful when assessed in combination with other measures of the microbial community.

Assessment of beta-diversity, in combination with analyses such as principal coordinates analysis, allows for determination of global differences between samples, and can be based on the presence or absence, or abundance, of particular organisms (e.g., Jaccard's dissimilarity, Bray–Curtis metrics), as well as phylogeny (evolutionary relationships between microbes; e.g., UniFrac).^{79,80} This has also led to the identification of characteristic microbial communities within the human population, termed enterotypes, which have been broadly linked to long-term dietary intake.⁸¹ The presence of such differing community structures may be useful for stratification of patient groups and identification of individuals likely to respond to dietary interventions. However, the microbial networks and interactions, such as cross-feeding and competition that drive assembly of these communities, as well as the implications for nutrient utilisation, metabolite production and human health, remain to be completely understood. In this context, a focus on guilds or networks of co-abundant organisms that respond to the same dietary substrates, may be informative.⁸²

Importance of microbial function

A consideration of taxonomy alone does not provide mechanistic insight into the effect of diet on the microbial utilisation of nutrients. This is because the metabolic functional properties of microbes are not necessarily linked to taxonomic identity, as per the ecological principles of functional redundancy and strain variation. Functional redundancy refers to taxonomically distinct organisms that possess similar metabolic properties. For example, distantly related *Bifidobacterium adolescentis* (Actinobacteria phylum) and *Ruminococcus bromii* (Firmicutes phylum) both have the ability to degrade and utilise resistant starch.^{83,84} Therefore, it is unsurprising that an increased faecal SCFA concentration in response to resistant starch supplementation is associated with a greater abundance (relative or absolute) of different organisms across individuals.^{83,85} By contrast, strain variation refers to closely related microbes with vastly different functional capabilities, as a result of varying gene content.⁸⁶ This is highly relevant in the context of the GI microbiota, as exemplified by *Escherichia coli*, for which there are both probiotic and pathogenic strains.

The direct measurement of metabolites in combination with characterising microbial profile may be more informative for health outcomes, enhancing understanding of how microbes respond to habitual diet and dietary interventions. A focus on specific microbial metabolites such as by-products

of fibre fermentation (e.g., SCFA), choline metabolism (e.g., trimethylamine/trimethylamine *N*-oxide) and sulphur-containing compounds (e.g., hydrogen sulphide) has been extremely valuable for discovering associations between diet and clinical endpoints, such as intestinal barrier function or metabolic and cardiovascular health, and, in combination with microbial profiling, can explain inter-individual responses to dietary intake.^{87,88} Metagenomic analysis assesses microbial gene content, and therefore provides not only information on taxonomic composition but, also some inference of microbial metabolic activity. Although metatranscriptomic analysis provides a direct assessment of microbial transcriptional activity, the very short half-life of microbial RNAs means that this is highly confounded by timing of sample collection.⁸⁹ Metagenomic and metabolite analyses are also constrained by the volume of microbial 'dark matter' that still exists. Many organisms remain uncultured and lack sequenced genomes, and many (possibly up to 70%) genes, proteins and metabolites have unknown functions.^{90,91} The generation of readily accessible, integrated data that captures the metabolic properties and activity of the microbiome, especially where relevant to nutrient utilisation, will represent a key step forward in the field.

Analysis challenges

Collinearity

The issue of collinearity pervades both diet and microbial datasets. For diet, this is the notion that changing one component of the diet precipitates compensatory changes in others. This is less relevant in nutrient supplementation studies but is important in food or whole diet interventions. For example, a whole diet intervention aiming to modulate the microbiome through increasing intake of fruit, vegetables and wholegrain foods will likely result in a compensatory reduction in protein and/or fat intake, assuming that energy intake remains constant. This change in protein and/or fat intake will in itself have distinct effects on the microbiome. As an added complexity, these dietary changes will lead to higher fibre, polyphenol⁹² and unsaturated fatty acid intake,⁹³ each of which specifically impact the microbiome. Hence, collinearity limits the degree to which specific microbial changes can be attributed to altered intake of individual nutrients in most dietary intervention trials. Despite this, comprehensive assessment of all relevant nutrients will assist in the interpretation of findings. Three-dimensional modelling, or 'nutritional geometry' to simultaneously assess combinations of dietary components may also prove useful in deciphering some of the complexity of diet–microbiome interactions in human studies.⁹⁴

Sequence-based microbiome profiling studies, and other 'omics datasets, also result in large collinear datasets. This presents a variety of statistical challenges, and highlights the importance of involvement of an experienced biostatistician. Many commonly used statistical methods are not designed for proportional data.⁹⁵ There has been considerable

TABLE 2 Best-practice guidelines for diet–microbiome research

Study design

- Hypothesis-driven (design all aspects of study to answer a question regarding a specific diet–microbiome interaction)
- Power calculations; use largest sample size possible

Participant selection and characterisation

- Thoroughly characterised study participants (e.g., anthropometric data, disease status and severity, medications, lifestyle factors)
- Consider whether any factors warrant exclusion (e.g., recent antibiotic use)

Dietary data collection

- Utilise expertise of a nutrition professional
- Consider strengths and limitations of dietary assessment methods relevant to the research questions (see Table 1)
- Align dietary data collection with microbiota sampling (e.g., one day prior to stool collection)
- Employ strategies to reduce under-reporting and other recall errors

Biological sample collection

- Select region of GI tract appropriate to research question (e.g., stool versus mucosa, small versus large intestine)
- Align collection of microbiota and other biological samples (e.g., plasma)
- Transport and store samples appropriately (preservative or cold temperature)
- Ensure procedures are standardised for all participants to avoid methodological-induced variation
- Collect multiple samples to address intra-individual temporal variability

Dietary analysis

- Utilise expertise of a nutrition professional
- Single nutrient versus global (e.g., diet quality) approach
- Quantification of microbiota-accessible components (e.g., types of dietary fibre)
- Collinearity (consider nutritional geometry)

Microbial community analysis

- Develop analysis strategy with input of microbiome expert prior to commencing study
- Consistency across all aspects of sample processing and analysis (e.g., DNA extraction protocols; inclusion of controls to account for reagent contamination and batch effects; consistent use of bioinformatics pipeline)
- Select analysis method based on research question (e.g., metagenomic sequencing to identify capacity to utilise particular nutrients; direct measurement of metabolites)
- Consider multiple methods of microbial analysis (e.g., microbial profiling with metabolite quantification)
- Measure and report both relative and absolute abundance (e.g., using qPCR)
- Consider the impacts of intra- and inter-individual variability when designing analysis strategy

Statistical analysis

- Utilise expertise of an experienced bioinformatician for integration and analysis of diet–microbiome data
- Data proportional / not normally distributed: transform data (e.g., centred-log ratio)
- Sparse data (multiple zero datapoints): use statistical methods validated for microbiota data
- Where there are many more outcome measures than number of samples: apply stringent correction for multiple comparisons
- Multi-omics data: integrate datasets prior to analysis; use of modelling
- Confounding effects: multivariate/mixed models
- Utilise metrics and statistical tests developed specifically for microbiome analysis

Abbreviation: DNA, deoxyribonucleic acid; GI, gastrointestinal; qPCR, quantitative polymerase chain reaction.

debate over the normalisation strategies that should be applied to overcome this.^{95–97} Importantly, data normalisation/transformation should be applied, such as centred-log ratio, when utilising parametric statistical tests. In addition, specific metrics developed for analysis of sequence-based microbial profiling data are available to assess changes in abundance,^{98,99} correlations,^{100,101} or to identify biomarkers,¹⁰² and can complement standard statistical tests. When combining multiple large datasets (multi-omics), including dietary data, the statistical challenges are even more complex; however, method development is progressing in this area.¹⁰³

Type-1 error and power calculations

Another major analysis challenge in cross-sectional studies is the presence of type-1 errors that result when a large number of outcome measures (e.g., hundreds of bacteria, multiple dietary variables) are derived from a small number

of study participants. Correction for multiple comparisons, using false discovery rate or Bonferroni correction, can reduce the risk of type-1 error, although even these may be suboptimal as a result of the non-parametric and non-Gaussian distribution of microbiome data.¹⁰⁴ Hence, care must be taken not to over-interpret the significance of changes in abundance of individual OTUs/ASVs. Further complicating data analysis is the presence of ‘rare microbes’ that are present in some individuals but absent in the majority (i.e. zero-inflated data), increasing the risk of type-1 error⁹⁷; however, stringent data filtering methods (e.g., only considering microbes present in at least 25% of participants) can help alleviate this problem.

Background dietary change during intervention trials is also a common problem when interpreting diet–microbiome findings. In well-powered studies, randomisation theoretically controls for this. However, under-powering is common in microbiome-targeted supplementation research, and

lack of dietary assessment creates uncertainty over whether microbiome findings are indeed a result of the effect of supplementation *per se* or, instead, are influenced by change in background diet secondary to the Hawthorne effect or other unintentional variability in dietary intake. More participants clearly provide enhanced study power, although the numbers required even for small effect sizes in diet-microbiome research readily reach thousands because of the inter-individual variability in the baseline gut microbiome.⁷⁶ Although there has been exploration of how to apply power calculations in microbiome profiling studies,^{105,106} this is highly challenging when considering the microbiome as a complex ecosystem and the multivariate nature of datasets generated. Longitudinal studies provide a means to address some aspects of inter-individual variation, although many of the same statistical challenges remain. This highlights the utility of placing datasets from individual studies in context, such as through meta-analysis, to delineate trends from type-1 errors.^{79,107}

Recommendations for best-practice study design

Despite the challenges of diet-microbiome research, best-practice study design (Table 2, Figure 2) can aid in overcoming many of the aforementioned challenges. Hypothesis-driven approaches are key, where studies are designed to answer specific questions regarding host-diet-microbe interactions or interventions are tested in specific contexts. Overall trial design and planning for congruence in diet and biological sample collection, along with a consideration of the analysis and statistical challenges highlighted above, will facilitate high-quality research and greater confidence in reported outcomes.

The study population

Clearly, characterising the study population is fundamental in diet-microbiome research. Confounding factors are particularly important to measure, some of which exert a stronger influence on microbial profiles than host genetics.¹⁰⁸ These factors broadly include ethnicity, anthropometric data, health status, primary disease and comorbidities, medication use, and lifestyle factors,¹⁰⁸⁻¹¹¹ and may also serve as exclusion criteria, such as the recent use of antibiotics or probiotics. The microbiome composition in individuals with chronic disease, often characterised by reduced microbiome richness (such as in inflammatory bowel disease)¹¹² or temporal instability (such as in irritable bowel syndrome),¹¹³ is often not mirrored in healthy individuals, who have relative stability of the microbiota over time.¹¹⁴ In disease, and particularly in GI disorders, altered dietary habits may partially mediate this divergent response.¹¹⁵ Hence, perhaps one of the most important characteristics to measure and report is participant disease status (e.g., relapse/remission) and severity.

Trial design

There are three major possible types of trial design in diet-microbiome research and, as in any other field, the choice of design should primarily be guided by the research hypothesis. First, cross-sectional studies measuring diet and the microbiome at one point in time allow for evaluation of the associations between these two variables. These studies are highly subject to external confounding and, although they have an implicit inability to establish whether the condition (microbiome profile) or the exposure (diet) came first, they are important in helping generate testable hypotheses.^{2,3,116} Second, longitudinal studies that repeatedly measure diet and microbiome over time can provide deeper insight into diet-microbiome relationships because they allow measurement of the effect of temporal dietary variation on microbial composition and stability within an individual.⁹ Third, intervention studies are those that supplement or restrict a nutrient, food or food component, or implement a 'whole diet' alteration (e.g., Mediterranean or Atkins diet) and are therefore able to demonstrate more direct influences of dietary variables on the microbiome. Confounding effects of habitual diet and baseline microbial communities remain a challenge in intervention studies; however, effects will become more completely understood as further RCTs recruit participants based on pre-defined background diet,¹¹⁷ and we benefit from learnings of relevant longitudinal data.

Data collection: Diet

Timing dietary assessment so that it is immediately adjacent to biological sample collection is extremely important given the rapid effects of diet and other host factors on microbiome composition.^{1,4,9} Of the multiple methods used to assess diet (Table 1), the 24-h recall or unweighed food record are typically used to measure short-term intake. Reducing the number of recording days may ease participant burden, although this must be balanced against the importance of capturing daily dietary variation, which is of particular importance in diet-microbiome research.^{118,119} Importantly, short-term dietary data can also be used to confirm dietary composition remains stable throughout microbiome-targeted (e.g., probiotic or prebiotic) supplementation studies. Remarkably, assessment of diet is frequently overlooked in such trials, as highlighted in recent systematic reviews of fibre⁶¹ and probiotic supplementation.¹²⁰

Assessment of long-term dietary intake is also relevant, particularly in cross-sectional studies, and provides a broader nutritional context for how diet shapes overall community structure. Food frequency questionnaires (FFQ) are typically used for this, and nutrient-specific FFQs are also available, including some with high relevance to the microbiome, such as those measuring intake of prebiotic carbohydrates.¹²¹ Validated culturally-specific questionnaires are also available, enabling capture of aspects of diet unique to

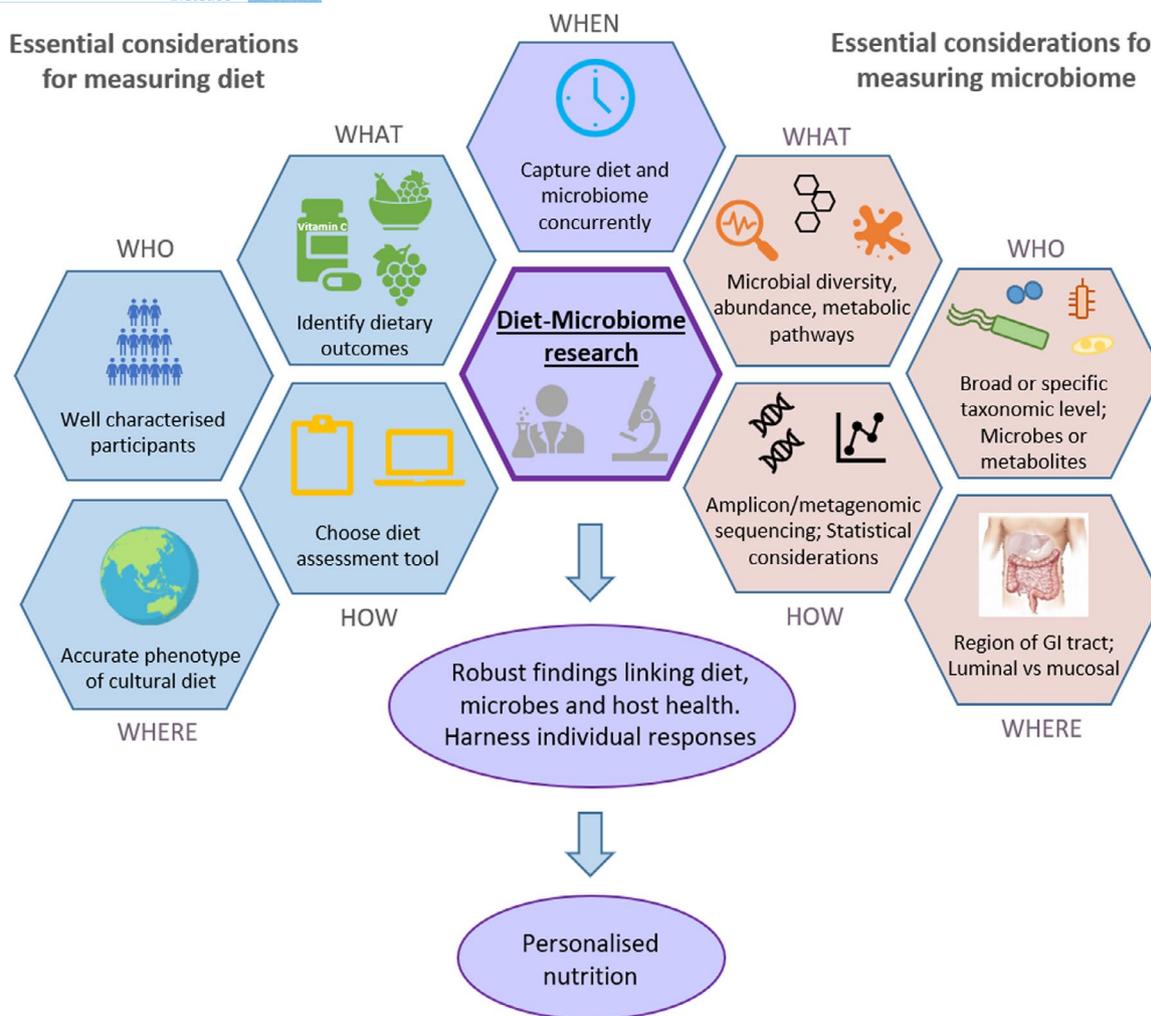


FIGURE 2 Summary of key considerations in diet–microbiome research. A robust study design includes a well characterised participant group, consideration of the availability of dietary substrates to microbes within intestinal niches, alignment of diet and microbiome data collection, rigorous choices of dietary and microbial assessment methods informed by experts, and appropriate statistical integration of these collinear datasets. GI, gastrointestinal

specific geographic regions. Broader measures of dietary composition, such as diet quality (Table 1), may also be applied as measures of long-term dietary intake^{122,123} and, in contrast to measuring nutrients alone, provide a global assessment of diet as previously described. In addition to cross-sectional research, habitual long-term intake may also be of relevance in intervention studies as mentioned previously, helping to explain inter-individual variation in microbial response to certain dietary interventions.¹¹⁷

Data collection: Microbiome

Collection of samples for microbiome analyses and related downstream processes including storage, processing and analysis methods, should be consistently applied across all samples to avoid technical variation.¹²⁴ Although time for biological sample transport and storage prior to analysis is required, care should be taken to limit sample degradation by using timely and appropriate preservation methods.¹²⁵

If a large number of samples need to be processed in batches, or after different lengths of storage, controls (such as mock microbial communities) should be included to account for batch effects and accounted for during statistical analysis.¹²⁶

As highlighted previously, the selection of suitable experimental analysis techniques for microbial samples, as well as the statistical approaches to be used and the metrics or outcomes to be reported, is crucial, and can potentially elevate (or appropriately downgrade) the significance of research outcomes. Ideally, a variety of microbiome analysis methods should be employed in concert with best-practice data analysis (Table 2), and these choices must be considered at the trial design phase prior to participant recruitment and sample collection. Example strategies could include complementary use of 16S rRNA gene amplicon sequencing for microbial profiling along with metabolite detection in the stool and blood⁸⁷; or the addition of metagenomic sequencing to facilitate assessment of how functional redundancy shapes individual responses.⁶⁰

CONCLUSIONS

A strong body of evidence exists regarding the importance of diet-microbiome interactions in facilitating health, or driving disease pathophysiology. However, the field continues to be dominated by a wealth of associative studies, fewer intervention trials and a lack of research providing mechanistic insights that identify the distinct diet-driven microbial alterations beneficial for human health. From a dietary perspective, tools that facilitate less onerous yet accurate estimations of dietary intake will be key. There is a need for the further development of methods that enable the measurement of nutrients in foods that are relevant to microbes (such as types of fibre and polyphenols), methods that estimate the availability of nutrients at the luminal substrate-microbe interface, and cost-effective methods that identify valid biological biomarkers of nutrient intake. From a microbiome perspective, improvements in our ability to measure and understand microbial metabolic properties, the response to nutrient availability, and interactions between microbes that drive community structure and function will move the field beyond taxonomic lists. Studies that investigate human diet-microbiome interactions using best-practice techniques, with collaboration across the disciplines of nutrition science and microbiology, are essential (Figure 2); this will drive research and clinical practice towards the goal of personalised nutrition recommendations for disease prevention, as well as the development of therapeutic diets for a broad spectrum of microbiome-associated disorders.

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CONFLICT OF INTERESTS

The authors have no conflicts of interest.

AUTHOR CONTRIBUTIONS

HS conceived the manuscript. All authors contributed equally to manuscript preparation and editing before submission.

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