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# Effect of Low Methionine Formula on Levels of IL-1β Serum and IL-1β Gene Expression in Knee Joint Cartilage Tissues of Normal Rabbits and ACL Induction OA Models

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**ABSTRACT**

Methionine deficiency is an environmental factor can degrade the quality of the bone, cartilage and modulate chondrocytes to increase protease secretion and change gene expression.The aim of the study was to determine the effect of methionine deficiency on IL-1β serum, expression of IL-1β in cartilage tissue of knee joints of New Zealand rabbit’s (*Oryctologus cuniculus*). The experimental animals were divided into 6 treatment groups: normal group with the addition of DL-methionine 0.25%, normal group with the addition of DL-methionine 0.15%, normal group with the addition of DL-methionine 0.00%, ACL group with the addition of DL-methionine 0.25%, ACL group with the addition of DL-methionine 0.15%, ACL group with an addition of 0.0%. Examination of IL-1β serum by ELISA with RayBio Rabbit commercial kit. Examination of IL-1β expression immunohistochemically using IL-1β anti-rabbit primary antibody with a Santa Cruz commercial kit (Sc7884). The results were analysed using one way ANOVA, followed by LSD. The results indicate that methionine deficiency (DL-methionine 0.0%) is able to increase IL-1β serum. The expression of IL-1β in knee joint cartilage tissue appears to be increased significantly through the metabolic effects or interactions with biomechanical changes. Methionine deficiency has the same ability in normal and pathological conditions, has a tendency to increase IL-1β serum and IL-1β gene expression in joint cartilage tissue.

***Keywords:*** *Methionine deficiency, Il-1β serum, IL-1β expression, Knee joints cartilage*

#### INTRODUCTION

Methionine as an essential amino acid in the body’s metabolic cycle has the ability at several control points including in protein synthesis, DNA / RNA synthesis, genetic expression, trans-methylation and fat, carbohydrate metabolism(1). Inadequate nutritional intake when conditions require high nutrient levels will sharply increase the risk of micronutrient deficiency

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and can interfere with growth in adolescence and will increase the risk of degenerative diseases in old age(2).

One of the degenerative diseases is osteoarthritis (OA) which can cause chronic disability and have serious health impacts, especially in the elderly(3). Osteoarthritis is a multifactorial disease caused by genetic and non-genetic risk factors or environmental factors such as age, obesity, injury, mechanics, metabolic disorders and endocrine. Through different molecular and cellular mechanisms, various risk factors cause changes in the expression of cytokine, proteinase, extracellular matrix proteins in cartilage to form the pathogenesis of osteoarthritis(4-7). Interleukin- 1β (IL-1β) is the main pro-inflammatory cytokine that plays a role in catabolic processes that induce cartilage

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damage, decrease proteoglycan synthesis, collagen and increase aggrecan secretion and stimulate chondrocytes to produce matrix metalloproteinase (MMPs) enzymes.

(8*)* IL-1β adheres to the receptor on the surface of chondrocytes and synoviocytes causing transcription of MMPs genes so that enzyme production increases(9)*.* Increased production of MMPs, especially MMP-13, can mediate the degradation of type II collagen and aggrecan proteoglycans(10,11).

Methionine is known as precursor in the formation of cysteine was the main source of sulphate for sulphatation reactions for synthesis of glycosaminoglycan, proteoglycan(12). Sulfation of extracellular matrix macromolecules is very important to maintain the quality of cartilage. The loss of extracellular matrix proteoglycans is an early sign of osteoarthritis.

Decreased proteoglycans cause the extracellular matrix to dehydrate, decrease the ability to withstand loads. This causes chondrocytes to increase protease secretion and degeneration of cartilage tissue. The hypothesis of this study is that methionine deficiency can increase IL-1β serum and increase IL-1β expression in cartilage of the knee joint of normal rabbit and rabbit model OA.

#### MATERIALS AND METHOD

###### Methionine deficiency formula

The methionine deficiency formula was prepared at the Food Technology Laboratory of the Health Department of Malang. It was formulated using a mixture of local food ingredients in the same amount and composition as cornmeal, soy flour, polar, vegetable oil and salt, minerals, vitamins. Methionine used in the form of DL-methionine and added to the formula with a dose of 0.25%, 0.15% and 0.0% per 1 kg of formula. The nutrient contain of the three DL-methionine formulas is the same, protein (12.9%), fat (8.7%) and carbohydrates (65.4%).

###### Animals Model OA

Female, white, 4-6 months old, New Zealand rabbits (*Oryctologus cuniculuc*) from Modern Rabbit Farming of Batu Animal Husbandry Department were used as an animal model ACL. All procedures were performed on the approved research protocol by the Ethics Committee of Faculty of Medicine, Universitas

Brawijaya (Number:372/EC/KEPK/09/2016). As an Anterior Cruciate ligament incision model (ACL) conducted by a team of veterinary surgeons from Animal Clinic of Central Animal Husbandry Training (BBPP), Center Batu, East Java.

###### Design

The rabbits were divided into 6 groups: normal rabbits with DL-methionine 0.25%; normal rabbits with DL-methionine 0.15%; normal rabbits with DL- methionine 0.0%, ACTL rabbit normal rabbits with DL- methionine 0.25%; normal rabbits with DL-methionine 0.15%, 0.15% DL-methionine 0.0%.

###### Measurement of intake

DL-methionine intake was measured every day (g/day/rabbit). It was calculated by dividing the total intake by 35 days.

###### IL-1β serum

IL-1β serum was measured using ELISA, and performed according to commercial kit instruction (Ray Bio Rabbit IL-1β, ELL-IL-1β). A 50µ blank and standard solution were put into empty wells. A total 50μl of serum each sample and put into the wells and incubated in at 37oC, covered with thin-foil wrap for 30 minutes. The solution was rinsed 4 time with PBST, added 50 secondary antibody conjugated with HRP and incubated once more in 370C, covered with wrap for 30 minutes. After 15 minutes of 370C incubation stop solution (in NaOH) was added and ELISA plate was read at 450 nm wavelength.

###### The expression of IL-1β

Immunohistochemistry was used to measure IL- 1β expression in joint cartilage. Using a commercial kit (Santa Cruz ((Sc7884)) with polyclonal anti-rabbit primary antibody IL-1β. The procedure following the manufacturer’s protocol. Observation of chondrocytes was carried out using a BX51 (Olympus) microscope on 400 x objective magnification.

###### Statistic analysis

All data obtained were expressed as mean ± standard deviation, then analyzed using ANOVA and Post Hoc test with LSD test.

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###### FINDINGS

**Intake of Methionine Formula**

In normal rabbits the average food intake was different between treatments, low methionine intake was more than other treatments. Induction of ACL intake low methionine did not increase food intake, intake of food was lower although statistically insignificant (table 1).

###### Table 1. Mean Intake of Methionine Formula per day

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| DL- metionine | n | Intake of Normal Rabbit | p-value | Intake of ACL Rabbit | p-value |
| Mean ± SD\*(g/day) | Mean ± SD\*(g/day ) |
| DL-metionin 0.25% per 100g | 4 | 60.20 ± 1.73a | 0.00 | 76.86 ± 0.82 | 0.14 |
| DL-metionin 0.15% per 100g | 4 | 66.59 ± 2.46b | 74.12 ± 2.76 |
| DL-metionin 0.0% per100 g | 4 | 77.67 ± 0.94c | 73.58 ± 2.59 |
| Note: \*Ducan test results show if mean ± SD there are different letters then there is a meaningful difference and if it contains thesame letter there is no difference. |

IL-1β Serum

In general, there was no difference in serum IL-1β levels between treatments. Although it was not statistically significant, low methionine intake in normal rabbits had a slightly higher IL-1β serum level compared to other rabbits. Induction of ACL increases serum ILl-1β levels.

###### Table 2. Concentration of IL-1β serum of rabbits

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Formula of methionin** | **n** | **IL-1β concentration (µg/dl)****Rabbit Normal** | **Sig** | **IL-1β concentration (µg/dl)****Rabbit ACL** | **Sig\*** |
| **Mean ± SD\*** | **Mean ± SD** |
| DL-methionine 0.25% per 100 g | 4 | 0.20 ± 0.10a | 0.00 | 0.23 ± 0.04a | 0.00 |
| DL-methionine 0.15% per100 g | 4 | 0.15 ± 0.00b | 0.14 ± 0.03b |
| DL-methionine 0.0% per 100 g | 4 | 0.22 ± 0.01c | 0.24 ± 0.02a |
| Note: \**One way Anova* results, followed by post hoc test using *LSD*a,b,cdifferent letters indicate a significant difference. |

**Expression of IL-1 β**

Expressions of IL-1β in cartilage of the knee joint between treatments was a significant differences. Low methionine intake in normal rabbits IL-1β expression

was higher than other rabbits. ACL induction increases IL-1β expression in cartilage of the knee joint, although it was not statistically significant.

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###### Table 3. Average IL-1β expression in rabbit knee joint cartilage tissues

**Formula DL- methionine n**

**Expression of**

**IL-1β normal rabbit p-value**

**Expression of**

**IL-1β ACL rabbit p-value**

**Mean ± SD\* Mean ± SD\***

DL-methionine 0.25% per 100g

DL-methionine

4 3.75 ± 1.50a

6.25 ± 0.96a

0.15% per 100g

DL-methionine 0.0% per 100 g

4 12.25 ± 0.96b 9.00 ± 0.82b

0.01

4 15.25 ± 0.96c 15.75 ± 1.26c

0.00

Note: \*One way Anova results, followed by post hoc test using LSD

a,b,cdifferent letters indicate a significant difference.

1 2 3

B


### 1 2 3

Note 1:

1. The preparation of cartilage of the rabbit's knee joint is normal
2. Cartilage preparation of ACL rabbit incision knee joints Note 2:
	1. DL-methionine formula 0.25%
	2. DL-methionine formula 0.15%
	3. DL-methionine 0.0%

**Figure 1. Expression of IL-1β by immunohistochemical method (400 x enlargement) appears brownish in the cytoplasm of cells,**

**using stained DAB.**

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#### DISCUSSION

Nutritional deficiency or imbalance of nutrients is one of the environmental factors that can affect cartilage health. Methionine as an essential amino acid is known to have a very important role for growth and development(13). Several studies have proven that reducing methionine in the diet can prolong life span, induce changes in energy metabolism, weight loss(14). In the group of normal rabbits which were given a low methionine formula, more food intake was compared to the control rabbit group (DL-methionine 0.25%). In another study the same results, in adult mice that were given methionine (DL-methionine 0.0%) diet for 6 months increased food intake. According to Hasek, animals fed a low methionine diet would consume more food than animals fed a control diet (0.86% methionine)

(15).

An imbalance of amino acids can cause a reduction in the flexibility of the food consumed. According to Harper, methionine is one of the amino acids that has the ability to eat from other amino acids. So that deficiencies and excess of methionine intake have a large impact on food consumption. Several previous studies have shown that methionine restriction diets (DL-methionine 0.0%) can increase the flexibility of metabolism and energy use or glucose during normal conditions(15,16). So limiting methionine in normal conditions can increasing energy use and energy expenditure(17). Changes in plasma amino acid concentrations can physiologically contribute to conditions of malnutrition and inflammation, low methionine intake has been shown to be involved in inflammatory responses and oxidative stress(18). In the elderly, interleukin-1 plays a role in normal homeostasis and the inflammatory response that is responsible for the development of chronic diseases such as osteoarthritis(19). In this study giving a low methionine formula (DL-methionine 0.0%) gave a minimal effect on inflammation. Supported by several other studies that show that increased IL-1β in serum occurs in diseases associated with metabolic syndromes such as atherosclerosis, chronic heart failure and type 2 diabetes, 1 and autoimmune diseases and rheumatoid arthritis(20,21).

Interleukin -1β (IL-1β) has been known to be a major mediator of inflammation that damages joint cartilage. IL-1β is produced by inflammatory cells (lymphocytes, granulocytes, plasma cells) of the synovial membrane and by the chondrocytes themselves by autocrine or

paracrine. IL-1β works by binding to specific receptors on the cell membrane, initiating cascades that cause induction and increase or inhibit various immune responses(22). Interleukin-1β has the effect of increasing the secretion of MMPs including MMP-13, suppressing type II collagen synthesis, inhibiting TGF-β which serves to stimulate chondrocyte proliferation, matrix synthesis(18,23,24).

In this study normal rabbits which were given a low methionine formula had IL-1β expression in the knee joint cartilage higher than normal rabbits which were given enough methionine formula (DL-methionine 0.25%) (p <0.05). This shows that low methionine intake has the potential to initiate and improve the development of osteoarthritis. In the posttraumatic phase there is an increase in inflammatory mediators that cause acute inflammation resulting in homeostasis and metabolic imbalances(25).

Under conditions of low methionine intake, causing the synthesis of glycosaminoglycan (GAG) including proteoglycans, chondrocytin sulphate the extracellular matrix component of cartilage becomes obstructed(26,27). Reducing the synthesis of glycosaminoglycan or proteoglycans will change the structure and composition of extracellular matrices to cause changes in biosynthesis and chondrocyte activity which are the beginning in the pathogenesis of osteoarthritis*(28,29).* Interleukin -1β (IL-1β) can induce matrix metalloproteinase (MMP) enzymes which can degrade various components of extracellular matrix such as collagen, proteoglycans in physiological and pathological conditions(30). Chondrocytes secrete matrix metalloproteinase-13 (MMP-13) in response to interleukin-1 (IL-1).

###### Limitations

This study was carried out in the short term to determine the effect of intake of methionine deficiency on catabolic gene expression in normal knee joint cartilage tissue and the OA damage model was not significantly different. Further research is needed with a longer period of time and more samples to determine differences in the effects and mechanisms of methionine deficiency on the expression of catabolic and anabolic genes in knee joint cartilage in normal weight or obese human or experimental animal models.

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#### CONCLUSION

This study shows that methionine deficiency has the same ability in normal and pathological conditions, has a tendency to increase IL-1β serum and increase IL-1β gene expression in knee joint cartilage tissue.

###### ADDITIONAL INFORMATIONS

The funding of this research was obtained from the Ministry of Health of the Republic of Indonesia. Before the implementation in the field, ethical clearance was obtained from Ethical Committee of Brawijaya University, Number: 372/EC/KEPK/09/2016. There is no conflict of interest relating to this research activity.

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