SciMedCentral
Annals of Sports Medicine and Research

Research Article

Milk Casein Hydrolysate Alleviates Muscle Soreness and Fatigue after Downhill Walking Exercise in Middle-aged to Elderly Men

Masayo Iwasa[1], Wataru Aoi[1]*, Airi Nakayama[1], Sayori Wada[1], Hidetoshi Miyazaki[2], Teppei Nakamura[2], Kohji Ohki[2] and Akane Higashi[1]

[1]*Laboratory of Health Science, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Japan*
[2]*Microbiology & Fermentation Laboratory, Calpis Co., Ltd, Japan*

***Corresponding author**
Wataru Aoi, Laboratory of Health Science, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, 1–5 Hangi-cho Shimogamo, Sakyo-ku, Kyoto 606–8522, Japan, Tel/Fax: 81 75 703 5417; Email: waoi@kpu.ac.jp

Submitted: 08 August 2015
Accepted: 04 September 2015
Published: 05 September 2015
ISSN: 2379-0571

Copyright
© 2015 Aoi et al.

OPEN ACCESS

Keywords
- Milk casein hydrolysate
- Muscle damage
- Middle-aged to elderly men
- Downhill walking

Abstract

The purpose of this study was to investigate the effect of milk casein hydrolysate (MCH) supplementation on muscle damage and fatigue after acute low-intensity exercise in middle-aged to elderly men. Fourteen healthy middle-aged to elderly men participated in each of two trials of the study: exercise with placebo and exercise with MCH. Participants carried out downhill walking (decline grade: -5%, speed: 5 km/h, time: 30 min). Test tablet (placebo or MCH) was taken before and after exercise in double-blind method. Degree of fatigue, and biochemical and physiological parameters were examined during and after exercise. Immediately after exercise, fatigue-related psychological factors were significantly decreased in the intake of MCH, compared with the placebo in a visual analogue scale (p = 0.03) and profile of mood state (p = 0.04) tests. A tendency was shown for lower blood lactate values in the MCH trial, compared with the placebo trial (p = 0.08). On the day after exercise, muscle soreness was significantly suppressed by the intake of MCH, compared with the placebo (p = 0.03). These results suggest that MCH supplementation alleviates muscle soreness and fatigue induced by downhill walking in middle-aged to elderly men.

ABBREVIATIONS

CK: Creatine Kinase; MCH: Milk Casein Hydrolysate; POMS: Profile of Mood State; PWV: Pulse Wave Velocity; RPE: Rate of Perceived Exertion; VAS: Visual Analogue Scale; WHO: World Health Organization

INTRODUCTION

It is well known that the risk of metabolic diseases in middle-aged to elderly people is higher than that shown in younger people [1]. Daily exercise prevents and improves metabolic syndrome, a pre-disease state; thus, it leads to the prevention of non-communicable diseases [2,3]. Based on that background and considering that physical inactivity increases the risk of mortality and the development of diseases, the World Health Organization (WHO) announced global recommendation on physical activity for health, particularly for middle-aged to elderly people [4,5]. Walking is one of the major exercises recommended for middle-aged to elderly people, because it can be performed easily and safely. It has actually been reported that habitual walking prevented or improved cardiovascular diseases and overweight [6-8]. However, middle-aged to elderly people are more susceptible to muscle soreness and fatigue than young people [9,10]; which could cause a decrease in their motivation for exercise.

Unaccustomed and strenuous exercise causes muscle damage, which is clinically shown as muscle soreness and involves protein degradation and ultrastructural changes, which is often called “delayed-onset muscle damage”. Exercise-induced muscle damage can be caused by several factors, including mechanical stress, calcium accumulation, and oxidative stress [11-14]. It has been suggested that muscle functions, such as energy metabolism and power output, are difficult to maintain in damaged muscles

[15,16]. Previous studies have reported that glucose utilization as an energy substrate in the whole body is decreased in cases where muscle damage occurs after exercise, caused by an impairment of insulin-dependent glucose uptake in the damaged muscles [17,18].

It has been reported that oxidative stress and certain inflammatory cytokines impair glucose uptake via the inactivation of insulin signaling pathways in muscle cells [19-21]. The infiltration of phagocytes into damaged muscle tissue is observed after strenuous exercise and an inflammatory response is implicated in the development of delayed-onset muscle damage [12,22]. In addition, an elevation of the levels of oxidative damage in cellular components has also observed in damaged muscles [11,12]. Thus, inflammatory cytokines and oxidative stress can decrease insulin-dependent glucose uptake in exercise-induced damaged muscles [23,24]. Moreover, it has become clear that the levels of inflammatory cytokine and oxidative products in blood and various tissues are higher in middle-aged to elderly people [25]. Therefore, if muscle damage easily occurs even with low-intensity exercise, the improvement in glucose metabolism induced as an effect of exercise may be suppressed.

Fermented milk has several salutary effects, including prolonged lifespan, antihypertensive and immune system regulation [26-28]. Previously, in an animal study, we showed that *Lactobacillus helveticus*–fermented milk prevented muscle damage induced by acute exercise via the activation of antioxidative enzymes of skeletal muscle [29]. Furthermore, we found that the supplementation of *Lactobacillus helveticus*–fermented milk improved glucose metabolism and alleviated the effects of muscle soreness after high-intensity exercise in young healthy men [30]. However, it is unclear whether or not that effect is also shown in low intensity exercise, such as the exercise performed daily by middle-aged to elderly people. Moreover, it has been suggested that fermented milk contains several particular peptides and amino acids that could influence several physiological effects. Fermented milk is manufactured by fermenting skimmed milk with a starter culture. During this process, proteins contained in the milk casein are digested and converted into small peptides, which are more easily absorbed by the intestines, compared to amino acids or large oligopeptides [31]. However, *Lactobacillus helveticus*–fermented milk still contains much unhydrolysed casein, and the productivity of peptides by milk fermentation is limited. A new enzymatic method for manufacturing these peptides from casein was recently developed using an *Aspergillus oryzae* protease [32]. Milk casein hydrolysate (MCH) and *Lactobacillus helveticus*–fermented milk include common peptides [33], suggesting the possibility that the same effects will be acquired. Thus, the purpose of this study was to investigate the effect of MCH supplementation on muscle damage and fatigue after downhill walking exercise in middle-aged to elderly men.

MATERIALS AND METHODS

Participants

Fourteen healthy middle-aged to elderly men with no regular exercise regimen habits were recruited to participate in this study. The mean ± S.E. characteristics of the participants were as follows: Age, 56.6 ± 2.8 years; height, 168.9 ± 1.4 cm; body weight, 68.4 ± 1.9 kg; body mass index, 23.9 ± 0.5 kg/m^2; and body fat percentage, 22.6 ± 1.1%. All participants were free of the signs, symptoms, and history of any overt chronic diseases. None of the participants were currently taking any medications or dietary supplements. This study was approved by the Ethics Committee of Kyoto Prefectural University (No. 45), and all of the participants signed an informed consent form after reading about the design and protocol of the study.

Study design

All of the participants attended the two trials included in the study, exercise with placebo intake (placebo) and exercise with MCH intake (MCH) in a repeated-measures experimental design. These trials were performed in a random order using a counter-balanced design and were separated by at least two weeks for any individual participants in order to avoid biasing of the muscle damage. The participants were also asked to refrain from caffeine and alcohol ingestion 24 h before each trial and were asked not to eat or drink anything except for water from 22:00 on the night before the trial to the next morning. Dietary records on the day of the trial were performed to avoid significant differences of food intakes between placebo and MCH trials. In the first trial, an example of the recording method was shown to the participants beforehand, and they recorded dietary contents according to it. And, the recorded contents were repeated in the second trial.

Supplementary tablets

The MCH hydrolyzed casein was produced by proteolytic enzymes. MCH containing peptides were prepared according to a previously described procedure [32]. The powdered casein hydrolysate was punch-pressed into tablets after the addition of diluents, emulsifier, and lubricant (test sample). The amount of peptides in the test sample was measured by the liquid chromatography-mass spectrometry method as described in the previous report [34] with some modification. Val-Pro-Pro and Ile-Pro-Pro were quantified using the internal standard method with Val-Pro-Pro isotope (($^{13}C^5$) Val-($^{13}C^5$) Pro-Pro, m/z 324.2) and Ile-Pro-Pro isotope (Ile-($^{13}C^5$) Pro-Pro, m/z 332.2) obtained from SCRUM Inc. (Tokyo, Japan). The placebo employed was non-hydrolyzed casein, and it was also presented in the shape of a tablet (Table 1). Each of the participants consumed 2 tablets with a cup of water before and after the exercise, using a double-blinded method.

Experiment schedule

On the first experiment day of each trial, the participants ate breakfast at 7:30 and came to laboratory at 9:00, where they sat on a chair until the beginning of the exercise test. Subsequently, the test tablets (placebo or MCH) were consumed, and blood lactate and blood glucose were measured using the finger stick test method. Walking exercise was performed from 30 min after consumption of the test tablets. During the exercise, the heart rate and the rating of perceived exertion (RPE) - the Borg 15 points (6–20) scale, were measured. Immediately after the exercise, the blood lactate, blood glucose and fatigue grade were measured. Afterwards, the participants consumed the test tablets again at 30 min after the end of the exercise. On the second day of the

Table 1: Nutritional information of the test tablets in placebo and MCH conditions.		
	Placebo	**MCH**
Energy (kcal)	5.4	5.4
Protein (g)	0.4	0.3
Free amino acid (mg)	0.0	182
Peptide (mg)	0.0	154
Val-Pro-Pro (mg)	0.0	1.4
Ile-Pro-Pro (mg)	0.0	2.0
Fat (g)	0.04	0.04
Carbohydrate (g)	0.9	0.9
Sodium (mg)	5.0	5.0

Abbreviations: Values are represented as composition included in 4 tablets.

study, the participants ate breakfast (steamed rice, 200 g) at 7:30, and returned to the laboratory at 9:00. Subsequently, the body composition was measured and blood sample was collected. Then, blood pressure, heart rate, skin blood flow, and pulse wave velocity (PWV) measurements were conducted. Next, the degree of subjective muscle soreness was evaluated. A schematic illustration of the experimental schedule was shown in Figure 1.

Exercise procedure

After stretching, the participants performed walking exercise for 30 min. The exercise was performed on a downhill treadmill (My Mountain 5050, Tobeone Co., Ltd., Gyeonggi-do, Republic of Korea) at a 5% decline grade. Walking speed was increased to 5 km/h after a warming-up period at 3 km/h for 2 min, and it was maintained at that speed until the end of exercise. Heart rate and RPE were measured every 3 min during the exercise.

Fatigue grade

Two questionnaires using a visual analogue scale (VAS) and a profile of mood state (POMS) were used to evaluate the fatigue grade immediately after the exercise. Using the VAS, the participants were asked to indicate the intensity of perceived fatigue on a 100-mm horizontal line. The left side stated "having no fatigue", while the right side stated "having max fatigue". The POMS questionnaire consists of 65 items, providing answers ranging from 0 (not at all) to 4 (extremely). That can be consolidated into six mood scales: "tension-anxiety", "depression-dejection", "anger-hostility", "vigor", "fatigue", and "confusion".

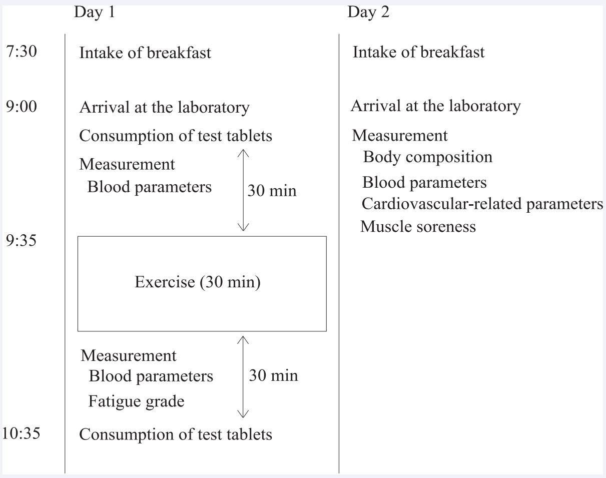

Figure 1 Schematic illustration of the randomized placebo-controlled double blinded trial schedule.

Muscle soreness

Subjective muscle soreness in the femoral, crural, and gluteus maximus muscles was evaluated by palpation and movement (bending and stretching) using the VAS. The participants were asked to indicate the intensity of perceived soreness for each muscle part on a 100-mm horizontal line. The left side stated "having no soreness", while the right side stated "having max soreness". The total soreness value was calculated by adding the soreness values for the 3 different muscles.

Blood parameters

Blood samples were collected from capillary using a finger stick test method. The separated plasma was stored at -80° until measurement of creatine kinase (CK) and insulin. The blood lactate and glucose were measured using simple measuring instruments (blood lactate: Lactate Pro, Arkray, Inc., Kyoto, Japan; glucose: Glu Test, Sanwa Kagaku Kenkyusho Co., Ltd., Aichi, Japan). CK and the insulin level in the plasma were measured by using an enzyme-linked immunosorbent assay kit (CK: Max Discovery™ Creatin kinase Enzymatic Assay kit, Bioo Scientific Co. TX, USA; insulin: Mercodia Ultrasensitive Human Insulin ELISA, Mercodia AB, Uppsala, Sweden).

Cardiovascular-related parameters

The blood pressure and heart rate were monitored with a humerus sphygmomanometer (EW3100, Panasonic Electric Works Co., Ltd., Osaka, Japan). The skin blood flow was measured with a laser doppler blood perfusion imager (PeriScan PIM 3 System, Integral Co., Tokyo, Japan). The PWV was calculated using a blood-pressure pulse wave inspection apparatus (FORM BP-203PRE, Omron Colin Co., Ltd., Tokyo, Japan) in the supine decubitus position.

Statistical analysis

All of the data were shown as the mean±standard error. The Wilcoxon signed-ranks test was used for data on blood lactate, plasma insulin and muscle soreness with a non-normal distribution, and the paired t-test was used for data on other parameters with a normal distribution to compare between conditions. The significance level was assumed to be 5%.

RESULTS

Heart rate and RPE

Heart rate gradually increased over time during the exercise, and reached 87.1 ± 2.5 beats/min (placebo) and 86.4 ± 2.9 beats/min (MCH) at the end of the exercise, when RPE showed scores of 11.5 ± 0.7 (placebo) and 11.4 ± 0.5 (MCH). No significant differences were found between the placebo and MCH consumption groups in either the heart rate ($p = 0.32$) or the RPE ($p = 0.45$) throughout the exercise, indicating that the same exercise load was applied to both conditions.

Fatigue grade

In the examination using VAS, the fatigue grade immediately after exercise showed a significantly low value for the MCH consumption group, compared with the placebo group (p = 0.03) (Table 2). Moreover, in the examination using POMS, the score of the fatigue-related item immediately after exercise was also significantly lower for the MCH consumption group, compared with the placebo group (p = 0.04) (Table 2). None of the other items in the POMS showed any significant change between the placebo and MCH consumption groups (data not shown).

Muscle soreness

On the next day of exercise, using the evaluation by palpation, the level of muscle soreness in the femoral and crural muscles was significantly suppressed in the MCH consumption group, compared with the placebo group (femoral muscle: p = 0.02, crural muscle: p = 0.04) (Table 3). The significant effect was not found in the gluteus maximus muscle (p = 0.24) (Table 3). The total score for muscle soreness in the three muscles was significantly suppressed in the MCH consumption group, compared with the placebo group (p = 0.03) (Table 3). In the evaluation by movement, the level of muscle soreness also showed a tendency to be suppressed in the MCH consumption group (date not shown). Significant difference in the level of muscle soreness was not observed between first and second trials, showing no repeat-bout effects.

Blood parameters

Immediately after the exercise, the blood lactate values showed a tendency for lower values in the MCH consumption group, compared with the placebo group (p = 0.08), although there was not significant difference (Table 4). The blood glucose values obtained immediately after exercise did not show any significant difference between the placebo and MCH consumption groups (p = 0.34) (Table 4). On the next day of exercise, no significant differences were found between the placebo and MCH consumption groups for the blood glucose, plasma CK, or plasma insulin (blood glucose: p = 0.29, plasma CK: p = 0.36, plasma insulin: p = 0.83) (Table 4).

Cardiovascular-related parameters

On the next day of exercise, no significant difference were shown in the systolic blood pressure and diastolic blood pressure between the placebo and MCH consumption groups (systolic blood pressure: p = 0.68, diastolic blood pressure: p = 0.18) (Table 5). The heart rate showed a tendency for lower value in the MCH consumption group, compared with placebo group (p = 0.08) (Table 5). No significant changes were shown in the skin blood flow or PWV between the placebo and MCH consumption groups (skin blood flow: p = 0.44, PWV: p = 0.32) (Table 5).

DISCUSSION

The present study revealed that the muscle soreness and

Table 2: Comparison of fatigue grade immediately after exercise.

	Placebo	MCH	*P* - value
VAS (score)	3.4 ± 0.5	2.5 ± 0.4	0.03
POMS (score)	6.3 ± 1.1	5.0 ± 1.0	0.04

Abbreviations: Values are represented as mean ± S.E. for 14 men.
Placebo, the trial of exercise with placebo intake; MCH, the trial of exercise with MCH intake; VAS, visual analog scale; POMS, profile of mood state.

Table 3: Comparison of muscle soreness on the next day of exercise.

	Placebo	MCH	*P* - value
Femoral muscle (score)	1.8 ± 0.6	1.1 ± 0.4	0.02
Crural muscle (score)	1.7 ± 0.6	1.4 ± 0.6	0.04
Gluteus maximus muscle (score)	1.2 ± 0.5	0.9 ± 0.3	0.24
Total score (score)	4.7 ± 1.5	3.4 ± 1.3	0.03

Abbreviations: Values are represented as mean ± S.E. for 14 men. The total score was calculated by adding the soreness values for the 3 different muscles. Placebo, the trial of exercise with placebo intake; MCH, the trial of exercise with MCH intake.

Table 4: Comparison of blood parameters after exercise.

	Placebo	MCH	*P* - value
Immediately after exercise			
Blood lactate (mmol/L)	1.6 ± 0.2	1.2 ± 0.1	0.08
Blood glucose (mg/dL)	88 ± 4	90 ± 3	0.34
The next day of exercise			
Blood glucose (mg/dL)	131 ± 9	127 ± 7	0.29
Plasma CK (IU/L)	147 ± 16	153 ± 8	0.36
Plasma insulin (μU/mL)	16 ± 3	20 ± 5	0.83

Abbreviations: Values are represented as mean ± S.E. for 14 men. Placebo, the trial of exercise with placebo intake; MCH, the trial of exercise with MCH intake; CK: Creatine kinase.

Table 5: Comparison of cardiovascular-related parameters on the next day of exercise.

	Placebo	MCH	*P* - value
Systolic blood pressure (mmHg)	116 ± 4	115 ± 4	0.68
Diastolic blood pressure (mmHg)	80 ± 3	78 ± 2	0.18
Heart rate (beats/min)	69 ± 2	67 ± 2	0.08
Blood skin flow (PU)	106 ± 6	106 ± 6	0.44
PWV (cm/s)	1,373 ± 53	1,358 ± 56	0.32

Abbreviations: Values are represented as mean ± S.E. for 14 men. Placebo, the trial of exercise with placebo intake; MCH, the trial of exercise with MCH intake; PWV, pulse wave velocity.

fatigue parameters observed after downhill walking exercise, although these parameters were mitigated by the intake of MCH, with pre- and post-exercise. To our knowledge, this study is the first research to show the effect of diet supplementation using milk-related peptides in middle-aged to elderly men. These results would help greatly to the improvement of the exercise habits of middle-aged to elderly men. Daily exercise is effective in the prevention and improvement of non-communicable diseases. Based on the research findings published by the WHO, and Lee et al. [35], more than 150 min a week of moderate exercise, equivalent to brisk walking, is enough to ensure that one does not fall into the "physical inactivity" category, which accounts for 35.2% of the population worldwide. According to the statistics published by the WHO, physical inactivity is a major cause of death and development of non-communicable diseases, and it corresponds to 9.4% of total death risk. Accordingly, the WHO recommends that adults aged 18–64 years should do at least 150 min of moderate-intensity aerobic physical activity each week and increase that amount to 300 min if possible [5]. However, the half of participants drops out exercise within 6 months from the beginning, as shown in Dishman's report [36]. In contrast to barriers for younger adults, the major barrier for the elderly is related to health-related concerns [37], and it is difficult for over 80% of elderly people to participate in exercise due to at least one physical or psychological barrier [38]. In addition, in an investigation of community-dwelling elderly people, health problems and pain were also suggested as the most common barrier to exercise [39]. Muscle soreness also continues after the next day of exercise, and thus may interfere with activities in daily life. Therefore, muscle soreness after exercise could be one of the factors preventing the habituation of exercise [40]. In this study, MCH suppressed muscle soreness and fatigue in middle-aged to elderly men, which may contribute to the habituation of exercise.

In the present study, the subjective fatigue grade immediately after downhill walking exercise showed lower values in the MCH intake group. Although fatigue induced by exercise is caused by various peripheral and psychological factors [41], intracellular acidosis could be a key role for muscle fatigue. Even minimal decrease in muscle pH interferes with cross-bridge binding and ATPase activity due to competitive binding and reduced enzyme function [42]. Decreased intracellular pH may also impair oxidative enzyme activity and may adversely affect ryanodine receptor function [43]. Lactic acid, a major source of protons, is rapidly produced by muscle contraction, lowering the pH and inhibiting muscle contraction. However, in the present study, the subjective fatigue grade caused by exercise was lowered in the MCH consumption group, compared with the placebo consumption. Furthermore, the blood lactate after exercise tended to show lower values by ingesting MCH. Thus, these observations suggest that the MCH intake suppressed the subjective fatigue grade after exercise, which could be mediated by improving muscular acidosis. Although detail mechanism is unclear, a possibility might be caused by improvement of peripheral blood flow after intake of MCH. In the previous animal study [44], milk-derived peptides increased concentrations of plasma nitrate and nitrite, which expands a diameter of an artery and increases a blood flow after oral administration. Therefore, MCH might elevate oxygen supply to muscle cells during exercise and induce a predominance of aerobic metabolism over anaerobic metabolism, which leads to the prevention of acidosis via suppression of lactic acid production.

It is well known that muscle soreness generally occurs as a part of delayed-onset muscle damage [45]. Therefore, MCH might reduce the degree of muscle soreness by suppressing the muscle damage. Delayed-onset muscle damage is caused by a variety of factors, but it is considered that oxidative stress and inflammatory cytokines are involved, at least to some extent. Indeed, oxidative stress and inflammatory cytokines also increase at the initiation and developmental stages of muscle damage [12,17]. Therefore, it may be considered that muscle soreness will also be reduced by suppressing oxidative stress and inflammatory cytokines. MCH contains specific peptides, such as Val-Pro-Pro and Ile-Pro-Pro, which are equivalent to *Lactobacillus helveticus*–fermented milk. In the study using rats [29], phagocyte infiltration and inflammatory cytokines expression, markers of inflammation in damaged muscles, were markedly reduced by the consumption of *Lactobacillus helveticus*–fermented milk. In addition, lipid peroxide levels were elevated after exercise, but the fermented milk intake significantly reduced this elevation. Moreover, fermented milk upregulates the expression of antioxidant enzymes, such as superoxide dismutase-2, catalase and glutathione S-transferase α-1. In addition, the level of heat shock protein 70, a chaperone protein that can function as a tissue repairing and anti-inflammatory agent was also elevated by the consumption of fermented milk. In our previous study [30], the level of oxygen radical absorbance capacity, which is an anti-oxidization index, was also suppressed by the ingestion of fermented milk. Therefore, these previous studies support our concept that the consumption of MCH reduced muscle damage through regulating the antioxidant capacity induced by casein-derived peptides, which might also contribute the suppression of metabolic impairment induced by muscle damage. In the present study, CK, a muscle damage marker, was not changed between conditions, which might be caused by characteristics of subjects such as lower inflammatory response in middle-aged and elderly people than young people [46]. It has been documented that a decrease in muscle content in type II fibers linked with aging [47] and type II fibers are more susceptible to muscle damage than type I fibers [48]. Lower levels of muscle damage response in the middle-aged and elderly people may be explained by their lower muscle content of type II fibers.

Previous studies showed that MCH containing antihypertensive peptides such as Val-Pro-Pro and Ile-Pro-Pro were able to improve human blood pressure and PWV by continuous ingestion [49]. However, the cardiovascular index was not changed by ingestion MCH in the present study, which might be affected by duration and amount of intake. These peptides increased concentrations of plasma nitrate and nitrite, which expands a diameter of an artery and increase a blood flow after a single oral administration to Wistar rats [44]. Therefore, this may occur in the present study during walking exercise, and affect a tendency for heart rate, although nitric oxide was not measured at that time.

Although the detailed mechanisms of the inhibitory effect of MCH on muscle soreness and fatigue remain unclear, small peptides present in MCH may be the causative agents. *Lactobacillus helveticus*–fermented milk is manufactured by fermenting skimmed milk with a starter culture containing *Lactobacillus helveticus*. During this process, the proteins in skimmed milk are digested by *Lactobacillus* and converted into small peptides. The small peptides containing MCH may also have additional physiological benefits aside from their use as a source of protein. Several studies have reported that the specific peptides contained in fermented milk have various salutary effects, including an antihypertensive effect and the attenuation of arterial dysfunction [33,44]. In addition to such peptides, amino acids are another possible ingredient contained in MCH that may be involved in the effect shown by the consumption of MCH, since the amount of amino acids in MCH is larger, compared with the placebo tablets. In future studies, we should attempt to identify the specific small peptides and amino acids in both MCH and fermented milk. Moreover, despite having only a very small amount of peptides and amino acids, MCH exerted an inhibitory effect on muscle soreness and fatigue. In previous studies, the peptides from a milk beverage were absorbed intact into the circulation [50], which suggests that the peptides might have a direct function in peripheral tissues. In addition, growing evidence has been shown that the supplementation of lactic acid bacteria can affect the immune system through improving the intestinal environment, such as the intestinal flora, immunocompetent cells and lactic acid bacteria [51]. Therefore, we should also examine not only the direct effect of amino acids and peptides on skeletal muscle after absorption, but also any indirect effects, such as the regulation of the intestinal bacteria and brain-gut interaction.

CONCLUSION

We found that MCH supplementation alleviated muscle soreness and fatigue induced by downhill walking in middle-aged to elderly men. MCH may be useful for persons who perform physical activity for health promotion. Further research is required to examine the detailed mechanisms of the effect of MCH in mitigating muscle damage, along with the benefit of MCH in different subjects and conditions.

ACKNOWLEDGEMENTS

This work was partly supported by JSPS KAKENHI: Grant-in-Aid for Scientific Research (B) Grant Number 25282199, Japan Society for the Promotion of Science, and Kyoto Prefectural University Academic Promotion Fund.

Conflict of Interest

This study was supported in part by a research fund from Calpis Co., Ltd.

REFERENCES

1. Sun SS, Sabo R, Arslanian S, Wu R, Sabo C. Age variation and sexual dimorphism in the sixteen diagnostic clusters of risk factors for the metabolic syndrome. J Public Health. 2012; 20: 487-497.
2. Lakka TA, Laaksonen DE. Physical activity in prevention and treatment of the metabolic syndrome. Appl Physiol Nutr Metab. 2007; 32: 76-88.
3. Donley DA, Fournier SB, Reger BL, De Vallance E, Bonner DE, Olfert IM, et al. Aerobic exercise training reduces arterial stiffness in metabolic syndrome. J Appl Physiol (1985). 2014; 116: 1396-1404.
4. WHO. Global strategy on diet, physical activity and health. 2004.
5. WHO. Global recommendations on physical activity for health. 2010.
6. Kraus WE, Houmard JA, Duscha BD, Knetzger KJ, Wharton MB, Mc Cartney JS, et al. Effects of the amount and intensity of exercise on plasma lipoproteins. N Engl J Med. 2002; 347: 1483-1492.
7. Leon AS, Sanchez OA. Response of blood lipids to exercise training alone or combined with dietary intervention. Med Sci Sports Exerc. 2001; 33: S502-515.
8. Walker KZ, Piers LS, Putt RS, Jones JA, O'Dea K. Effects of regular walking on cardiovascular risk factors and body composition in normoglycemic women and women with type 2 diabetes. Diabetes Care. 1999; 22: 555-561.
9. Bouzid MA, Hammouda O, Matran R, Robin S, Fabre C. Changes in oxidative stress markers and biological markers of muscle injury with aging at rest and in response to an exhaustive exercise. PloS One. 2014; 9: e90420.
10. Power GA, Dalton BH, Rice CL, Vandervoort AA. Power loss is greater following lengthening contractions in old versus young women. Age (Dordr). 2012; 34: 737-750.
11. Aoi W, Naito Y, Sakuma K, Kuchide M, Tokuda H, Maoka T, et al. Astaxanthin limits exercise-induced skeletal and cardiac muscle damage in mice. Antioxid Redox Signal. 2003; 5: 139-144.
12. Aoi W, Naito Y, Takanami Y, Kawai Y, Sakuma K, Ichikawa H, et al. Oxidative stress and delayed-onset muscle damage after exercise. Free Radic Biol Med. 2004; 37: 480-487.
13. Gissel H, Clausen T. Excitation-induced Ca^{2+} influx and skeletal muscle cell damage. Acta Physiol Scand. 2001; 171: 327-334.
14. Proske U, Morgan DL. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. J Physiol. 2001; 537: 333-345.
15. O'Reilly KP, Warhol MJ, Fielding RA, Frontera WR, Meredith CN, Evans WJ. Eccentric exercise-induced muscle damage impairs muscle glycogen repletion. J Appl Physiol (1985). 1987; 63: 252-256.

16. Powers SK, Jackson MJ. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiol Rev. 2008; 88: 1243-1276.

17. Aoi W, Naito Y, Tokuda H, Tanimura Y, Oya-Ito T, Yoshikawa T. Exercise-induced muscle damage impairs insulin signaling pathway associated with IRS-1 oxidative modification. Physiol Res. 2012; 61: 81-88.

18. Del Aguila LF, Krishnan RK, Ulbrecht JS, Farrell PA, Correll PH, Lang CH, et al. Muscle damage impairs insulin stimulation of IRS-1, PI 3-kinase, and Akt-kinase in human skeletal muscle. Am J Physiol Endocrinol Metab. 2000; 279: E206-212.

19. De Alvaro C, Teruel T, Hernandez R, Lorenzo M. Tumor necrosis factor alpha produces insulin resistance in skeletal muscle by activation of inhibitor kappa B kinase in a p38 MAPK-dependent manner. J Biol Chem. 2004; 279: 17070-17078.

20. Plomgaard P, Bouzakri K, Krogh-Madsen R, Mittendorfer B, Zierath JR, Pedersen BK. Tumor necrosis factor-alpha induces skeletal muscle insulin resistance in healthy human subjects via inhibition of Akt substrate 160 phosphorylation. Diabetes. 2005; 54: 2939-2945.

21. Rudich A, Kozlovsky N, Potashnik R, Bashan N. Oxidant stress reduces insulin responsiveness in 3T3-L1 adipocytes. Am J Physiol. 1997; 272: E935-940.

22. Tidball JG. Inflammatory cell response to acute muscle injury. Med Sci Sports Exerc. 1995; 27: 1022-1032.

23. Gordon PM, Liu D, Sartor MA, Iglay Reger HB, Pistilli EE, Gutmann L, et al. Resistance exercise training influences skeletal muscle immune activation: a microarray analysis. J Appl Physiol (1985). 2012; 112: 443-453.

24. Aoi W, Naito Y, Yoshikawa T. Role of oxidative stress in impaired insulin signaling associated with exercise-induced muscle damage. Free Radic Biol Med. 2013; 65: 1265-1272.

25. Wu J, Xia S, Kalionis B, Wan W, Sun T. The role of oxidative stress and inflammation in cardiovascular aging. Biomed Res Int. 2014; 2014: 615312.

26. Nakamura Y. Studies on anti-hypertensive peptides in milk fermented with Lactobacillus helveticus. Biosci Microflora. 2004; 23: 131-138.

27. Reddy GV, Shahani KM, Banerjee MR. Inhibitory effect of yogurt on Ehrlich Ascites tumor-cell proliferation. J Natl Cancer Inst. 1973; 50: 815-817.

28. Vinderola G, Matar C, Perdigón G. Milk fermentation products of L. helveticus R389 activate calcineurin as a signal to promote gut mucosal immunity. BMC Immunol. 2007; 8: 19.

29. Aoi W, Naito Y, Nakamura T, Akagiri S, Masuyama A, Takano T, et al. Inhibitory effect of fermented milk on delayed-onset muscle damage after exercise. J Nutr Biochem. 2007; 18: 140-145.

30. Iwasa M, Aoi W, Mune K, Yamauchi H, Furuta K, Sasaki S, et al. Fermented milk improves glucose metabolism in exercise-induced muscle damage in young healthy men. Nutr J. 2013; 12: 83.

31. Craft IL, Geddes D, Hyde CW, Wise IJ, Matthews DM. Absorption and malabsorption of glycine and glycine peptides in man. Gut. 1968; 9: 425-437.

32. Mizuno S, Nishimura S, Matsuura K, Gotou T, Yamamoto N. Release of short and proline-rich antihypertensive peptides from casein hydrolysate with an Aspergillus oryzae protease. J Dairy Sci. 2004; 87: 3183-3188.

33. Nakamura T, Mizutani J, Ohki K, Yamada K, Yamamoto N, Takeshi M, et al. Casein hydrolysate containing Val-Pro-Pro and Ile-Pro-Pro improves central blood pressure and arterial stiffness in hypertensive subjects: a randomized, double-blind, placebo-controlled trial. Atherosclerosis. 2011; 219: 298-303.

34. Matsuura K, Mizuno S, Nishimura S, Gotou T, Yamamoto N. Quantitative analysis of the antihypertensive peptides Val-Pro-Pro and Ile-Pro-Pro in casein hydrolyzate using an *Aspergillus oryzae* protease: an LC-MS method. Milchwissenschaft. 2005; 60: 24-27.

35. Lee IM, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. Lancet. 2012; 380: 219-229.

36. Dishman RK. Motivating older adults to exercise. South Med J. 1994; 87: S79-82.

37. Schutzer KA, Graves BS. Barriers and motivations to exercise in older adults. Pre Med. 2004; 39: 1056-1061.

38. O'Neill K, Reid G. Perceived barriers to physical activity by older adults. Can J Public Health. 1991; 82: 392-396.

39. Cohen-Mansfield J, Marx M, Guralnik J. Motivator and barriers to exercise in an older community-dwelling population. J Aging Phys Act. 2003; 11: 242-253.

40. Justine M, Azizan A, Hassan V, Salleh Z, Manaf H. Barriers to participation in physical activity and exercise among middle-aged and elderly individuals. Singapore Med J. 2013; 54: 581-586.

41. Finsterer J. Biomarkers of peripheral muscle fatigue during exercise. BMC Musculoskelet Disord. 2012; 13: 218.

42. Jones DA, Turner DL, McIntyre DB, Newham DJ. Energy turnover in relation to slowing of contractile properties during fatiguing contractions of the human anterior tibialis muscle. J Physiol. 2009; 587: 4329-4338.

43. Bellinger AM, Mongillo M, Marks AR. Stressed out: the skeletal muscle ryanodine receptor as a target of stress. J Clin Invest. 2008; 118: 445-453.

44. Nonaka A, Nakamura T, Hirota T, Matsushita A, Asakura M, Ohki K, et al. The milk-derived peptides Val-Pro-Pro and Ile-Pro-Pro attenuate arterial dysfunction in L-NAME-treated rats. Hypertens Res. 2014; 37: 703-707.

45. Lewis PB, Ruby D, Bush-Joseph CA. Muscle soreness and delayed-onset muscle soreness. Clin Sports Med. 2012; 31: 255-262.

46. Toft AD, Jensen LB, Bruunsgaard H, Ibfelt T, Halkjaer-Kristensen J, Febbraio M, et al. Cytokine response to eccentric exercise in young and elderly humans. Am J Physiol Cell Physiol. 2002; 283: C289-295.

47. Lavender AP, Nosaka K. Comparison between old and young men for changes in makers of muscle damage following voluntary eccentric exercise of the elbow flexors. Appl Physiol Nutr Metab. 2006; 31: 218-225.

48. Monemi M, Eriksson PO, Eriksson A, Thornell LE. Adverse changes in fibre type composition of the human masseter versus biceps brachii muscle during aging. J Neurol Sci. 1998; 154: 35-48.

49. Cicero AF, Rosticci M, Gerocarni B, Bacchelli S, Veronesi M, Strocchi E, et al. Lactotripeptides effect on office and 24-h ambulatory blood pressure, blood pressure stress response, pulse wave velocity and cardiac output in patients with high-normal blood pressure or first-degree hypertension: a randomized double-blind clinical trial. Hypertens Res. 2011; 34: 1035-1040.

50. Foltz M, Meynen EE, Bianco V, Van Platerink C, Koning TM, Kloek J. Angiotensin converting enzyme inhibitory peptides from a lactotripeptide-enriched milk beverage are absorbed intact into the circulation. J Nutr. 2007; 137: 953-958.

51. Gourbeyre P, Denery S, Bodinier M. Probiotics, prebiotics, and synbiotics: impact on the gut immune system and allergic reactions. J Leukoc Biol. 2011; 89: 685-695.

Cite this article

Iwasa M, Aoi W, Nakayama A, Wada S, Miyazaki H, et al. (2015) Milk Casein Hydrolysate Alleviates Muscle Soreness and Fatigue after Downhill Walking Exercise in Middle-aged to Elderly Men. Ann Sports Med Res 2(8): 1045.