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RESEARCH ARTICLE

The effects of endurance physical exercise with fat-free milk intake as a therapy for metabolic syndrome and/or sarcopenia

Tetsuo Yamada[1*], Masaki Okada[2], Masami Matsuzaki[1,4], Akira Tanaka[3,5]

[1]Department of Nutrition and Dietetics, Kanto Gakuin University, Yokohama, Japan
[2]Department of Health and Nutrition Sciences, Komazawa Women's University, Inagi, Japan
[3]Professor emeritus at Kagawa Nutrition University, Sakado, Japan
[4]Department of Nutritional Management, Hana Professional Training of College, Tokyo, Japan
[5]Kichijoji Futaba Professional and Vocational College of Culinary Nutrition, Tokyo, Japan

*tyyamada@kanto-gakuin.ac.jp

ABSTRACT

Background: Nutrition and exercise are important factors for addressing metabolic syndrome and sarcopenia as they are related to insulin secretion. Proteins, particularly branched-chain amino acids (BCAAs), have insulinotropic effects, while exercise reduces insulin secretion. It is thus important to investigate the effects of protein intake and exercise alone and in combination to determine suitable dietary and exercise therapies for treating metabolic syndrome and sarcopenia.

Methods: Eight healthy young adult female volunteers participated in a crossover trial consisting of two 5-day experiments. Days 1 and 2 comprised a body-weight-maintained adjustment period during which the participants consumed control diets (energy, 2,010 kcal; protein, 51.9 g). Days 3 to 5 comprised the treatment period during which the participants consumed experimental diets (energy, 2,010 kcal; protein 82.3 g) containing 402 kcal of fat-free milk, and either performed only normal daily activities (non-Ex) or performed normal daily activities and exercised on a bicycle with an ergometer at a target intensity of about 50% of the maximal oxygen intake, expending 402 kcal of additional energy (Ex). Total urine samples were collected during the daytime (6:45 to 18:45) and nighttime (18:45 to 6:45 the next morning). Fasting blood samples were collected early in the morning before and after the treatment period.

Results: Plasma valine, leucine, and BCAA levels were significantly elevated after both the non-Ex and Ex periods. Serum insulin levels were significantly elevated only after the non-Ex period. Urinary C-peptide immunoreactivity excretion levels increased significantly during the non-Ex period, but they decreased significantly during the Ex period. After the Ex period, the serum triglyceride and remnant lipoprotein-cholesterol (RLP-C) levels were significantly decreased. Homeostatic model assessment for insulin resistance (HOMA-IR) and atherosclerosis index (AI) values were slightly, yet significantly, increased after the non-Ex period, but were unchanged after the Ex period. The degree of change (Δ) in the RLP-C level significantly and positively correlated with the Δ HOMA-IR and Δ AI.

Conclusions: Exercise attenuated the insulinotropic effects of protein intake and had beneficial effects on glucose and lipid metabolism. Thus, protein intake in conjunction with exercise is recommended for preventing and/or improving metabolic syndrome and sarcopenia.

1. Introduction

Metabolic syndrome and sarcopenia, as well as sarcopenic obesity, which is a combination thereof, are major challenges for maintaining and promoting health. Nutrition and exercise are both important factors for addressing metabolic syndrome and sarcopenia. For addressing metabolic syndrome, energy intake restriction is effective,[1] and obesity, impaired glucose and lipid metabolism can be alleviated through aerobic exercise, which directly promotes the consumption of glucose and free fatty acids (FFAs).[2-4] In contrast, for addressing sarcopenia, an adequate addition of energy is needed, in addition to an adequate supply of nutrients, such as proteins and vitamin D.[5] In terms of exercise for preventing sarcopenia, a relatively large mechanical load is effective for increasing muscle mass, and as a result, glucose and lipid metabolism are also indirectly improved.[6]

Insulin lowers the blood glucose (BG) level and stimulates protein anabolism. From the perspective of metabolic syndrome, it is necessary to avoid the excessive secretion of insulin. However, insulin is also considered to contribute to muscle mass increases.[7] The following relationships can be seen among nutrition, exercise, insulin secretion, and body protein anabolism. Proteins, particularly branched-chain amino acids (BCAAs), are utilized for energy,[8] protein maintenance, and anabolism,[9,10] but they simultaneously have insulinotropic effects.[11-13] On the other hand, exercise reduces insulin secretion and promotes body protein anabolism.[14-16] Thus, although the intake of BCAAs and exercise have opposite effects on insulin secretion, they both affect body protein anabolism in the same way. Therefore, when considering dietary and exercise therapy for treating metabolic syndrome and sarcopenia, it is important to investigate the effects of protein intake and exercise alone and in combination. In addition, it is necessary to consider the effects of increased insulin secretion on glucose and lipid metabolism.

We hypothesized that the intake of protein containing BCAAs would increase muscle protein synthesis and suppress muscle protein breakdown, and that exercise would have beneficial effects on the BCAA intake-induced insulin secretion, and glucose and lipid metabolism. In the present study, we examined the effects of the intake of BCAA-rich fat-free milk in combination with or without exercise on protein-related parameters, including plasma BCAA levels, muscle protein synthesis- and breakdown-related parameters, and glucose and lipid metabolism-related parameters, including the serum insulin level, urinary C-peptide immunoreactivity (CPR) excretion level, homeostatic model assessment for insulin resistance (HOMA-IR), and the atherosclerosis index (AI), to assess the effectiveness of dietary and exercise therapies against metabolic syndrome and sarcopenia.

2. Materials and Methods

2.1 Subjects

Eight healthy young adult female volunteers (age, 20 ± 1 years (mean ± standard deviation); body mass index, 20.7 ± 3.9 kg/m^2) participated in the metabolic experiment. A subject briefing session was held to explain the methods of the current study, including the 10-day restriction of usual life activities, dietary control, and exercise load. Consequently, eight subjects were enrolled, which was the small number needed for the statistical analysis. G*Power 3.1 was

used for power analysis,[17,18] and the power for detecting differences was calculated using the alpha level (0.05), sample size, and effect size (calculated from the means, standard deviations, and Spearman's correlation coefficients between pairs of variables). In our previous study with a sample size of 6, for the changes in the serum triglyceride (TG) level after exercise, the power for detecting differences was 85%.[19] In the present study, for the changes in the serum TG and remnant lipoprotein-cholesterol (RLP-C) levels after exercise, the power for detecting differences was 98% and more than 99%, respectively. Furthermore, it was more than 99% for the differences of the urinary CPR excretion level with or without exercise. The subjects provided written informed consent to participate in all procedures associated with the study, which adhered to the tenets of the Declaration of Helsinki.

2.2 Experimental procedures

The Research Ethics Committee of Kanto Gakuin University approved the study protocols (approval number: 2015-2-5).

2.2.1 Experimental protocol

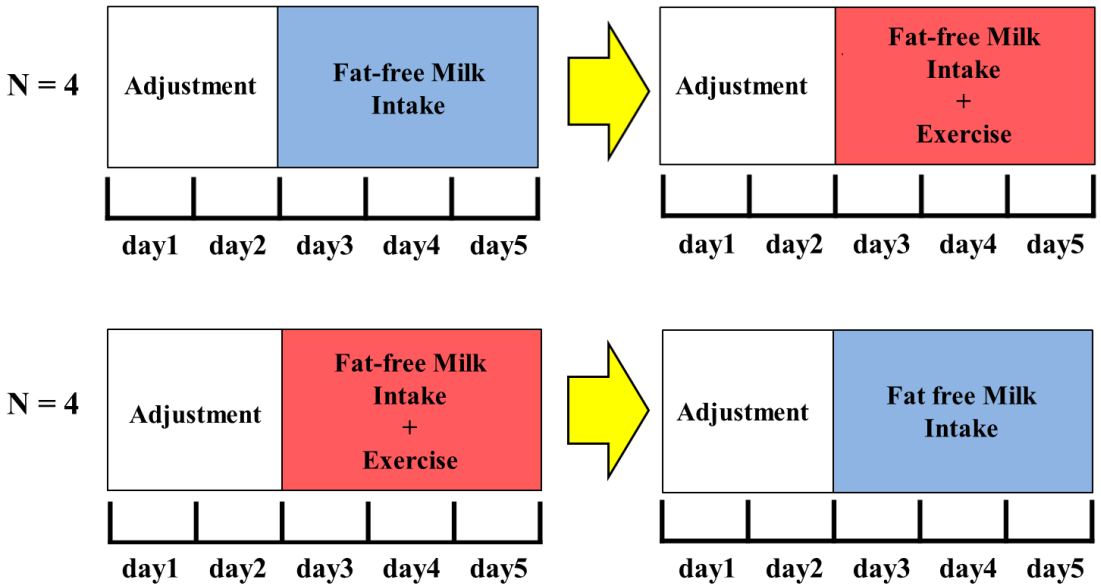
The study comprised two 5-day experiments (10 days in total; **Fig. 1**). All eight subjects participated in both experiments. Each experiment consisted of a 2-day body-weight-maintained adjustment period followed by a 3-day treatment period (5 days in total). In one of the two experiments, the intervention was fat-free milk intake with no exercise (non-Ex) during the 3-day treatment period, while in the other experiment, the intervention was fat-free milk intake with exercise (Ex) during the 3-day treatment period. After the end of the first 5-day experiment, there was a wash-out period of more than 10 days during which the subjects had access to an *ad libitum* diet before the second 5-day experiment started. The two experiments were performed with a crossover design, *i.e.*, the experiment with fat-free milk intake and non-Ex was followed by the experiment with fat-free milk intake and Ex for half of the participants, and the order was reversed for the other half of the participants.

For each experiment, the subjects were gathered at the university in the evening of the day before the start of the experiment. The experiment started at 06:30 on the first day. Throughout the experiment, the subjects remained at the university, and stayed in the metabolic experiment building.

During the adjustment period from day 1 to day 2, the subjects woke up at 06:30, performed the final urine collection for the nighttime before at 06:45, then began collecting all urine for the next 12 h from that point on as the daytime collections. Breakfast was served between 08:30 and 09:00, lunch was served between 13:00 and 13:30, and dinner was served between 18:00 and 18:30. The subjects performed the final urine collection for the daytime at 18:45, then began collecting all urine for the next 12 h from that point on as the nighttime collections. Other than taking a shower between 19:30 and 21:30, and going to bed at 22:00, they could spend their time freely in the metabolic experiment building doing as they pleased, except for eating and additional physical activity.

The experimental (non-Ex and Ex) periods lasted from day 3 to day 5. During the non-Ex period, the daily schedule remained unchanged from that of the adjustment period, and fat-free milk replaced one-fifth of the diet of the adjustment period in terms of the energy intake.

Fig. 1. Experimental protocol. After the end of the first 5-day experiment, there was more than 10-day wash-out period during which the subjects had access to an ad libitum diet before the second 5-day experiment started. Fasting blood samples were collected early in the morning on the first day of each treatment period (the 3rd day of each 5-day experiment) and at the end of each treatment period (the morning after day 5 of each 5-day experiment). Total urine samples were collected during daytime (6:45 to 18:45) and nighttime (18:45 to 6:45 the next morning) every day throughout each experiment.



During the Ex period, the daily schedule remained unchanged from that of the adjustment period, except for the addition of an exercise load that was performed from 10:15 in the morning and from 14:45 in the afternoon, and the diet was the same as that during the non-Ex period.

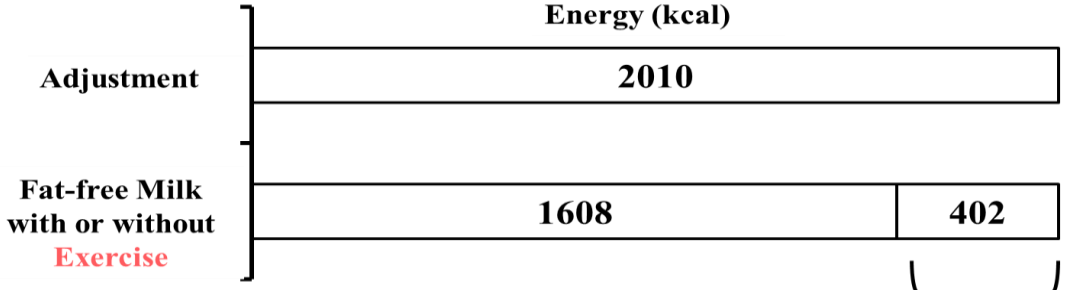
The room temperature during the experimental period was maintained at a dry bulb temperature of 25°C to 26°C, and at a wet bulb temperature of 22°C to 23°C.

2.2.2 Experimental diet

For the experimental diet, basically homogeneous foods (crackers, margarine, jam, omelet, tomato ketchup, vegetable juice, soy baked goods, biscuits, spaghetti, meat sauce, vitamin beverages, ice lollies, milled rice, mashed potatoes, miso, *Komachi-fu* (a gluten product), and soda) were used as ingredients. The experimental diet during the body-weight-maintained adjustment period was set to meet the recommended dietary allowance or adequate intake defined in the Dietary Reference Intakes for Japanese.[20] The calculated values per day on the Standard Tables of Food Composition in Japan were as follows: energy, 2,010 kcal; protein, 51.9 g; lipid, 51.8 g; carbohydrate, 325.9 g; and protein:fat:carbohydrate (PFC) ratio, 10.7:23.8:65.4.[21] For the diets of the non-Ex and Ex periods, one-fifth of the diet of the adjustment period (reduced by reducing all foods in proportion to the energy intake) was replaced by fat-free milk. Fat-free milk was consumed with each of the three meals every day, and its volume was determined by calculating the equivalent energy amount in 200 ml of whole

milk per meal, which was considered to be a normal amount of milk consumed in daily life. The calculated values per day were as follows: energy, 2,010 kcal; protein, 82.3 g; lipid, 42.1 g; carbohydrate, 318.7 g; and PFC ratio, 17.3:19.3: 63.4 (Fig. 2).

Fig. 2. Energy and composition of experimental diet.



	Adjustment	Fat-free Milk
Protein (g)	51.9	82.3
Lipid (g)	51.8	42.1
Carbohydrate (g)	325.9	318.7

402 kcal of additional energy was expended with Exercise.

2.2.3 Procedures for the exercise

Before the start of this study, an exercise tolerance test was performed involving gradually increasing work rates on a bicycle with an ergometer. The heart rate was recorded by telemetry (DS-3400; Fukuda Denshi Co., Ltd., Tokyo, Japan). Expired gas samples before and at a steady state during exercise were collected using a Douglas bag, and the gas volume was measured using a dry gas meter (DC-5A; Shinagawa Corporation, Tokyo, Japan). The expired oxygen and carbon dioxide concentrations were measured using an expired gas monitor (Portable Gas Monitor AR-1; Arco System Inc., Kashiwa, Japan). The relationships among the additional energy expenditure calculated based on oxygen intake and the respiratory exchange ratio, work rate (kilopond of the bicycle ergometer), and heart rate were determined for each subject.

In the exercise experiment, participants expended 402 kcal of additional energy by pedaling a bicycle with an ergometer at a target intensity of about 50% of the maximal oxygen intake for 104 ± 11 min split between the morning and afternoon.

2.3. Sample collection and measurements

Fasting blood samples were collected early in the morning on the first day of each treatment period (the 3rd day of each 5-day experiment) and at the end of each treatment period (the morning after day 5 of each 5-day experiment). Total urine samples were collected during the daytime (6:45 to 18:45) and nighttime (18:45 to 6:45 the next morning) every day throughout each experiment.

The blood and urine samples were sent to: BML, Inc. (Tokyo, Japan) for the measurement of the levels of BG, serum urea nitrogen (UN),

creatine kinase (CK), immunoreactive insulin (IRI), FFA, TG, total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), and high density lipoprotein-cholesterol (HDL-C); SRL, Inc. (Tokyo, Japan) for the measurement of the levels of plasma amino acids (valine, leucine, and isoleucine), serum insulin-like growth factor 1 (IGF-1), cortisol, RLP-C, urinary CPR, 3-methylhistidine (3-MH), and cortisol; and LSI Medience Corporation (Tokyo, Japan) for the measurement of the levels of urinary creatinine, UN, and three types of catecholamines (adrenaline, noradrenaline, and dopamine).

HOMA-IR was calculated by the formula [Fasting blood glucose level (mg/dl) × Fasting insulin level (μU/ml) / 405]. AI was calculated by the formula [(TC – HDL-C) level / HDL-C level].

2.4. Statistical analyses

The results are expressed as the median (range), because there was no guarantee that the data were normally distributed. All statistical analyses were performed using IBM SPSS Statistics 27 (IBM Corporation, Armonk, NY, USA). We applied the Wilcoxon signed-ranks test or Friedman's test to examine differences in the measures of central tendency, and evaluated the relationships between pairs of variables using the Spearman's correlation coefficient. Significance was established at $p < 0.05$ in all analyses.

3. Results

There was no significant difference in the timing of the menstrual cycle between the two experiments, i.e., when the length of the period from one menstruation to the next menstruation was taken as 100%, the first day of the non-exercise experiment was at the 68th ± 33rd percentile, and the first day of the exercise experiment was at the 68th ± 27th percentile.

3.1. Blood metabolic parameters

The changes in the blood levels of protein-related parameters (UN and BCAAs), muscle synthesis- and breakdown-related parameters (IGF-1, CK and cortisol), and glucose and lipid metabolism-related parameters are shown in **Table 1**.

The serum UN levels were significantly increased after both the non-Ex and Ex periods when compared to the adjustment period, and the degree of change (Δ) in the UN level (Δ UN) was significantly larger after the Ex period than after the non-Ex period. The plasma valine and leucine levels were significantly increased after both the non-Ex and Ex periods, with no significant differences in Δ valine and Δ leucine between the non-Ex and Ex periods. On the other hand, the plasma isoleucine levels were almost the same before and after the non-Ex period and the Ex period.

The serum IGF-1 levels remained unchanged after the non-Ex period, but decreased significantly after the Ex period, and the levels were significantly lower after the Ex period than after the non-Ex period. The serum CK levels were significantly higher after the Ex period than after the non-Ex period. The serum cortisol levels did not significantly change after either of the periods.

Table 1. Changes in the blood metabolic parameters.

Variable	non-Ex period			Ex period			Δ^1 vs Δ^2
	Before	After	Δ^1	Before	After	Δ^2	p -value
UN (mg/dl)	8.9 (6.7 to 12.2)	12.0 * (8.4 to 16.6)	+3.4 (+1.5 to +4.4)	8.5 (8.1 to 11.3)	13.2 *# (9.6 to 15.8)	+4.7 (+1.4 to +5.6)	**0.031**
Valine (nmol/ml)	172.2 (160.7 to 223.4)	194.1 * (169.4 to 245.5)	+16.6 (+8.5 to +76.9)	182.1 (145.4 to 208.4)	196.0 * (175.4 to 240.9)	+21.4 (+1.7 to +55.5)	0.742
Leucine (nmol/ml)	101.8 (94.3 to 124.2)	108.4 * (93.0 to 130.7)	+4.3 (−1.3 to +27.9)	98.8 (87.6 to 121.5)	106.1 * (96.0 to 129.2)	+8.2 (−2.8 to +22.7)	0.945
Isoleucine (nmol/ml)	55.0 (46.5 to 73.1)	52.7 (45.3 to 65.3)	−3.0 (−7.8 to +8.8)	55.2 (47.8 to 67.8)	56.9 (48.3 to 65.0)	+1.7 (−10.6 to +10.1)	0.742
BCAAs (nmol/ml)	332.6 (306.3 to 420.7)	355.1 * (307.7 to 439.2)	+17.8 (+1.4 to +109.6)	331.2 (280.8 to 397.7)	352.9 * (333.2 to 420.0)	+24.8 (−2.4 to +85.3)	0.844
IGF-1 (ng/ml)	260 (183 to 313)	263 (169 to 345)	−2 (−35 to +59)	244 (171 to 383)	226 *# (163 to 296)	−13 (−87 to +5)	0.219
CK (U/l)	68 (54 to 120)	68 (45 to 87)	−9 (−42 to +19)	74 (45 to 163)	93 # (54 to 124)	+11 (−65 to +57)	0.234
Cortisol (μg/dl)	9.81 (6.85 to 16.40)	9.24 (6.74 to 13.40)	+0.17 (−3.56 to +2.77)	10.50 (6.58 to 17.00)	9.38 (7.93 to 14.60)	−0.96 (−4.67 to +3.23)	0.641
BG (mg/dl)	84 (79 to 88)	84 (78 to 88)	+2 (−5 to +5)	82 (77 to 93)	82 (69 to 94)	−2 (−8 to +14)	0.656
IRI (μU/ml)	4.6 (3.2 to 8.6)	5.6 * (3.6 to 10.8)	+1.4 (−0.3 to +3.7)	5.2 (3.1 to 9.5)	4.0 # (2.2 to 10.1)	−0.8 (−2.9 to +4.8)	0.063
FFA (mEq/l)	0.42 (0.23 to 0.63)	0.52 * (0.33 to 0.92)	+0.09 (+0.03 to +0.29)	0.33 (0.18 to 0.74)	0.55 * (0.37 to 0.85)	+0.17 (+0.06 to +0.38)	0.313
TG (mg/dl)	90 (62 to 203)	90 (65 to 184)	−3 (−20 to +37)	98 (57 to 120)	73 *# (49 to 91)	−25 (−47 to −8)	**0.016**
RLP-C (mg/dl)	4.3 (1.9 to 10.7)	5.6 (1.9 to 9.4)	−0.0 (−1.3 to +2.0)	5.1 (3.0 to 6.5)	3.0 *# (2.3 to 5.5)	−1.4 (−2.8 to −0.5)	**0.008**
TC (mg/dl)	154 (117 to 217)	162 * (121 to 235)	+8 (−3 to +25)	156 (128 to 232)	154 (128 to 249)	+1 (−9 to +17)	0.219
LDL-C (mg/dl)	83 (62 to 144)	92 * (64 to 155)	+9 (−1 to +20)	80 (63 to 151)	87 * (72 to 173)	+12 (0 to +22)	0.461
HDL-C (mg/dl)	55 (47 to 63)	53 (44 to 69)	−3 (−3 to +6)	57 (47 to 74)	56 (48 to 73)	+1 (−5 to +2)	0.703

N = 8. Variables are given as the medians (minimum value to maximum value).

Δ^1, degree of change during non-Ex period; Δ^2, degree of change during Ex period.

* $p < 0.05$ compared to before the non-Ex period or the Ex period, # $p < 0.05$ compared to after the non-Ex period.

UN, urea nitrogen; BCAAs, branched chain amino acids; IGF-1, insulin-like growth factor 1; CK, creatine kinase; BG, blood glucose; IRI, immunoreactive insulin;

FFA, free fatty acid; TG, triglyceride; RLP-C, remnant lipoprotein-cholesterol; TC, total cholesterol; LDL-C, low density lipoprotein-cholesterol;

HDL-C, high density lipoprotein-cholesterol.

The BG levels remained fairly constant after the non-Ex and Ex periods. The serum IRI levels were significantly increased only after the non-Ex period, and were significantly lower after the Ex period than after the non-Ex period. The serum FFA levels were significantly increased after both the non-Ex and Ex periods, with no significant differences between the two periods. The serum TG and RLP-C levels remained unchanged after the non-Ex period,

but they significantly decreased after the Ex period, and the levels were significantly lower after the Ex period than after the non-Ex period. Furthermore, the Δ TG and Δ RLP-C (amount of decrease) were significantly larger during the Ex period than during the non-Ex period. The serum TC levels were significantly increased only after the non-Ex period. The serum LDL-C levels were significantly increased after both the non-Ex and Ex periods. On the other hand, the serum HDL-C levels did not significantly change after both the non-Ex and Ex periods.

Fig. 3 shows the changes in the HOMA-IR calculated by the following formula: [Fasting blood glucose level (mg/dl) × Fasting insulin level (μU/ml) / 405]. Fig. 4 shows the changes in the AI calculated by the following formula: [(TC – HDL-C) level / HDL-C level]. The HOMA-IR values, similar to the serum IRI levels, were significantly increased only after the non-Ex period, and the Δ HOMA-IR (amount of decrease) tended to be larger (p = 0.055) after the Ex period than after the non-Ex period. The AI values were also significantly increased only after the non-Ex period, and were significantly lower after the Ex period than after the non-Ex period; the Δ AI (amount of decrease) tended to be larger (p = 0.078) after the Ex period than after the non-Ex period.

Fig. 3. Changes in the homeostatic model assessment for insulin resistance (HOMA-IR) calculated by the formula [Fasting blood glucose level (mg/dl) × Fasting insulin level (μU/ml) / 405]. N = 8. Data are shown as the medians (minimum value – maximum value). * p < 0.05 compared to before the Non-Ex period or the Ex period.

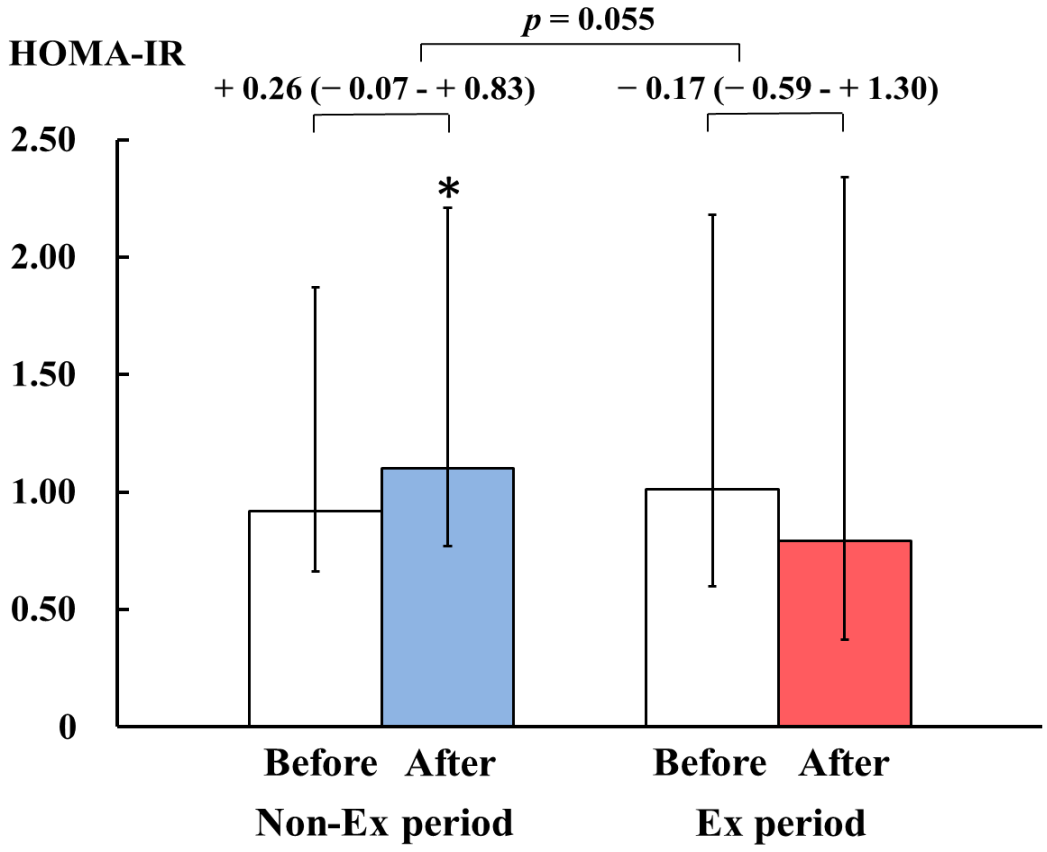
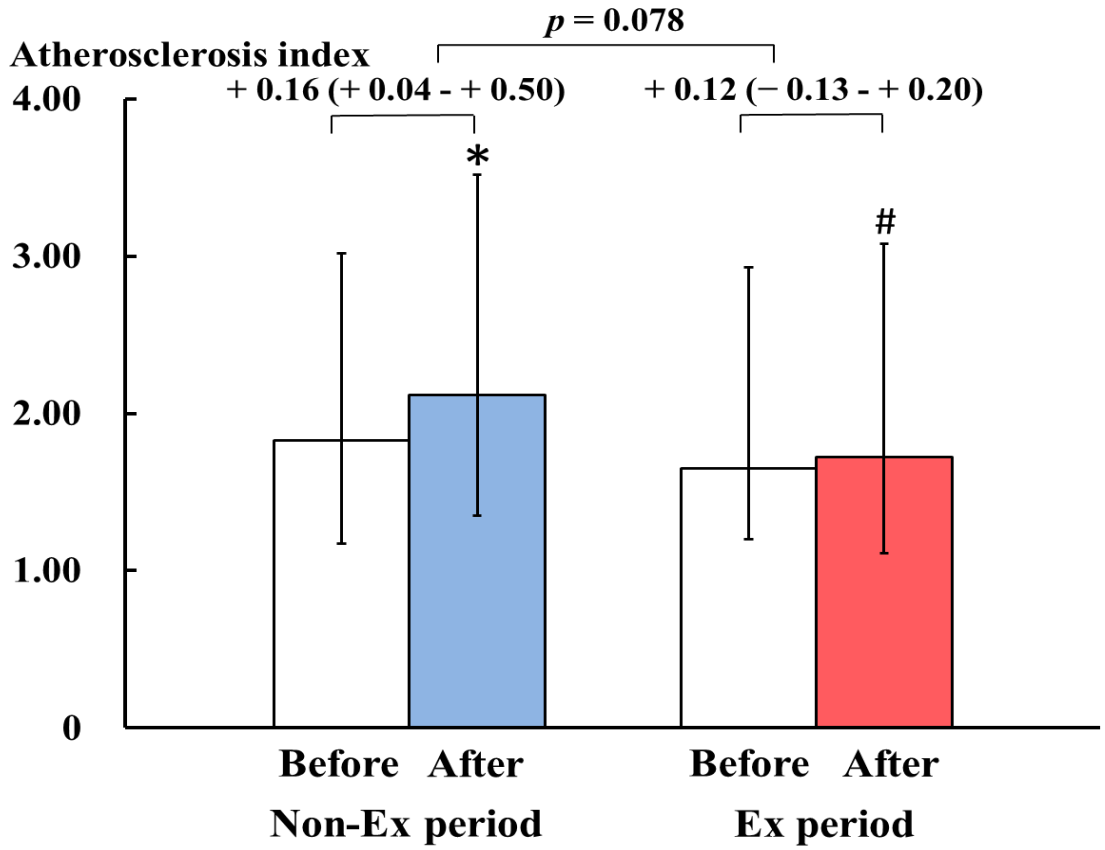


Fig. 4. Changes in the atherosclerosis index (AI) calculated by the formula [(TC – HDL-C) / HDL-C]. N = 8. Data are shown as the medians (minimum value – maximum value). * $p < 0.05$ compared to before the Non-Ex period or the Ex period.
# $p < 0.05$ compared to after the Non-Ex period.
TC, total cholesterol; HDL-C, high density lipoprotein-cholesterol.



3.2. Correlations among the glucose and lipid metabolism-related parameters

Spearman's correlation coefficients (the Δ during both the non-Ex and Ex periods (N = 16)) were calculated to examine the relationships among the glucose and lipid metabolism-related parameters (Table 2). The Δ HOMA-IR and Δ RLP-C were significantly positively correlated ($r = 0.509$, $p < 0.05$). The Δ TG was significantly positively correlated with the Δ RLP-C ($r = 0.875$, $p < 0.01$) and Δ AI ($r = 0.571$, $p < 0.05$), and negatively correlated with the Δ HDL-C ($r = - 0.718$, $p < 0.01$). The Δ RLP-C was significantly positively correlated with the Δ AI ($r = 0.570$, $p < 0.05$), and negatively correlated with the Δ HDL-C ($r = - 0.516$, $p < 0.05$). The Δ TC was significantly positively correlated with the Δ LDL-C ($r = 0.636$, $p < 0.01$) and Δ AI ($r = 0.621$, $p < 0.05$).

Table 2. Spearman's correlation coefficients among variables in terms of change (Δ) during both the non-exercise and the exercise periods (N=16).

	Δ HOMA-IR	Δ TG	Δ RLP-C	Δ TC	ΔLDL-C	Δ HDL-C	Δ AI
Δ HOMA-IR	—	0.398	**0.509***	0.124	– 0.220	– 0.162	0.105
Δ TG (mg/dl)		—	**0.875****	0.030	– 0.215	**– 0.718****	**0.571***
Δ RLP-C (mg/dl)			—	0.220	– 0.001	**– 0.516***	**0.570***
Δ TC (mg/dl)				—	**0.636****	0.492	**0.621***
Δ LDL-C (mg/dl)					—	0.435	0.356
Δ HDL-C (mg/dl)						—	– 0.270
Δ AI							—

* p<0.05, **p<0.01

HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; TG, serum triglyceride; RLP-C, remnant lipoprotein-cholesterol;

TC, total cholesterol; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol, AI (atherosclerosis index), (TC – HDL-C) / HDL-C ratio.

Although not shown in the table, with the Δ urinary CPR excretion level (daytime), the Δ TG ($r = 0.539$, $p < 0.05$) and Δ RLP-C ($r = 0.662$, $p < 0.01$) were significantly positively correlated.

3.3. Urinary metabolic parameters

The daily changes and the pooled mean data (the daytime (from 6:45 to 18:45), nighttime (from 18:45 to 6:45 the next morning), and daily totals) for the excretion levels of urinary creatinine, UN, CPR, 3-MH, catecholamines (adrenaline, noradrenaline, dopamine), and cortisol are shown in **Table 3**, and the Δ CPR is shown in **Fig. 5**.

The urinary creatinine excretion levels remained fairly constant during both the non-Ex and Ex periods, with no significant difference between the two periods. The urinary UN excretion levels increased significantly in the daytime, nighttime, and daily totals during both the non-Ex and Ex periods with fat-free milk intake, and there was no significant difference between the two periods. The urinary CPR excretion levels increased significantly in the daytime and daily totals during the non-Ex period, but they decreased significantly in the daytime during the Ex period, and were significantly lower during the Ex period than during the non-Ex period. Furthermore, the Δ CPR (amount of decrease) in the daytime and daily totals were significantly larger during the Ex period than during the non-Ex period. The urinary 3-MH excretion levels decreased significantly in the nighttime during the non-Ex period, while they remained unchanged during the Ex period. Regarding catecholamines, the urinary

adrenaline and noradrenaline excretion levels were significantly higher during the Ex period than during the non-Ex period. On the other hand, the dopamine excretion levels decreased significantly in the nighttime during the non-Ex period. The urinary cortisol excretion levels remained almost unchanged.

Table 3. Changes in the excretion levels of urinary metabolic parameters.

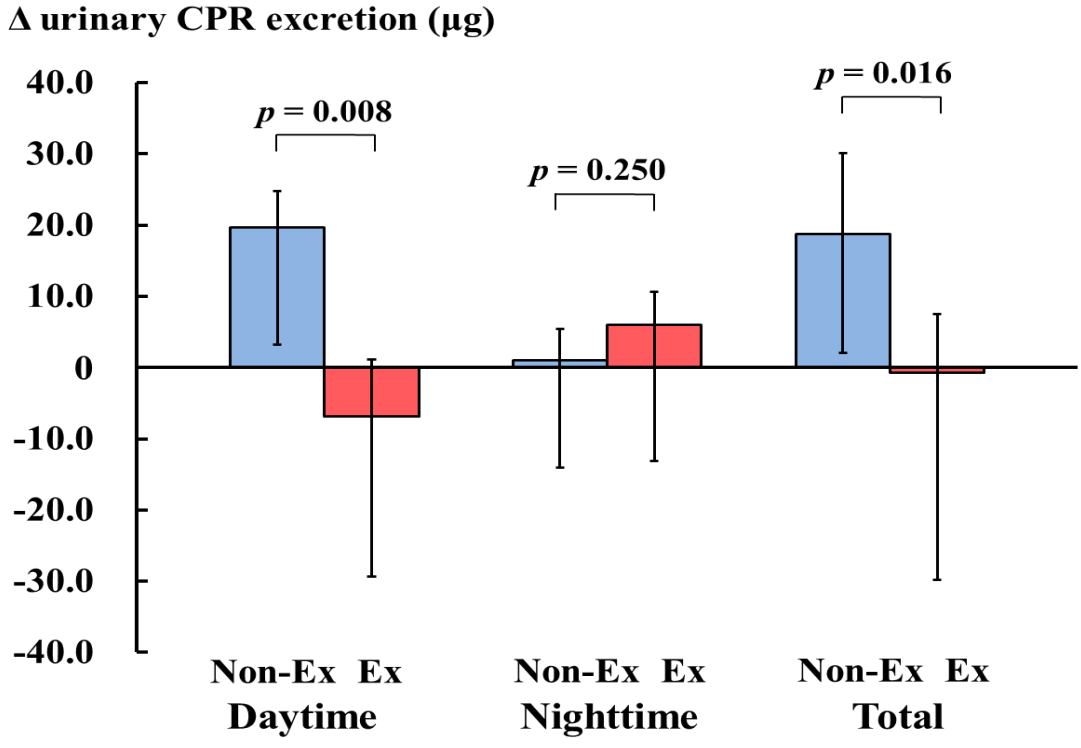
			Adjustment		Fat-free milk intake			Friedman's test (Day 2-5) p-value	Pooled mean
			Day 1	Day 2	Day 3	Day 4	Day 5		Day 3-5
Creatinine (mg)	Daytime	non-Ex period	485 (437 to 574)	501 (463 to 668)	533 (432 to 638)	525 (419 to 628)	537 (456 to 578)	0.622	533 (436 to 602)
		Ex period	543 (439 to 627)	524 (416 to 636)	531 (438 to 610)	470 (367 to 595)	518 (442 to 584)	0.140	502 (447 to 597)
	Nighttime	non-Ex period	520 (404 to 592)	505 (425 to 569)	446 (280 to 543)	491 (440 to 571)	471 (326 to 589)	0.094	461 * (397 to 551)
		Ex period	455 (375 to 549)	484 # (372 to 562)	460 (366 to 515)	486 (355 to 549)	474 (392 to 516)	0.278	483 (371 to 518)
	Total	non-Ex period	1041 (841 to 1115)	1035 (890 to 1237)	941 (842 to 1131)	1036 (858 to 1199)	1016 (782 to 1128)	0.191	977 (832 to 1153)
		Ex period	1004 (814 to 1176)	1010 (788 to 1198)	992 (823 to 1125)	947 (793 to 1118)	997 (843 to 1100)	0.191	987 (820 to 1114)
UN (mg)	Daytime	non-Ex period	3461 (2513 to 4049)	2977 (2326 to 3845)	3932 (3254 to 4458)	4414 (3636 to 4972)	4721 (4087 to 5052)	**< 0.001**	4364 * (3704 to 4806)
		Ex period	3481 (2577 to 3878)	2892 (2181 to 3404)	3645 (2956 to 4131)	4141 (2947 to 4799)	4692 (4038 to 5195)	**< 0.001**	4183 * (3515 to 4708)
	Nighttime	non-Ex period	2845 (2328 to 3647)	2823 (2289 to 3344)	3726 (2031 to 4789)	3912 (3355 to 4879)	3750 (2763 to 4905)	**< 0.001**	3959 * (3036 to 4400)
		Ex period	2412 (2253 to 2814)	2400 # (2039 to 2632)	3898 (2132 to 4203)	4265 (3744 to 4916)	4497 (3937 to 5132)	**< 0.001**	4178 * (3474 to 4750)
	Total	non-Ex period	6317 (5271 to 7265)	5597 (4728 to 6832)	7508 (6293 to 8980)	8281 (6991 to 9850)	8320 (6986 to 9957)	**< 0.001**	8224 * (6799 to 9003)
		Ex period	5899 (4994 to 6693)	5140 (4684 to 6021)	7672 (5088 to 8009)	8539 (7134 to 9081)	9149 (7975 to 10129)	**< 0.001**	8377 * (6989 to 8938)
CPR (μg)	Daytime	non-Ex period	43.6 (21.7 to 79.0)	44.3 (31.6 to 60.3)	60.3 (42.5 to 77.2)	60.3 (46.9 to 82.8)	70.6 (37.9 to 84.5)	**< 0.001**	64.9 * (42.4 to 81.5)
		Ex period	45.2 (27.0 to 80.0)	45.0 (30.9 to 81.6)	39.3 (23.8 to 59.7)	38.3 (24.2 to 52.3)	45.0 (29.0 to 57.9)	**0.043**	42.3 *# (27.4 to 52.2)
	Nighttime	non-Ex period	31.1 (14.8 to 41.1)	33.6 (17.1 to 41.5)	33.9 (8.1 to 53.9)	31.1 (16.0 to 48.4)	30.7 (15.1 to 46.3)	0.837	31.8 (13.1 to 46.9)
		Ex period	27.7 (17.6 to 36.1)	26.4 (16.7 to 39.3)	30.2 (8.8 to 52.6)	32.5 (23.6 to 45.2)	32.4 (16.6 to 39.6)	0.117	31.8 (16.3 to 43.1)
	Total	non-Ex period	72.5 (36.6 to 120.0)	77.8 (51.7 to 101.5)	94.6 (53.6 to 123.7)	96.4 (68.4 to 131.3)	98.1 (63.8 to 130.2)	**< 0.001**	98.8 * (63.0 to 128.4)
		Ex period	70.5 (50.6 to 114.8)	71.5 (47.5 to 120.9)	69.5 (42.4 to 111.6)	74.2 (47.8 to 95.2)	77.4 (45.7 to 97.5)	0.817	74.1 # (45.3 to 95.4)

			Adjustment		Fat-free milk intake			Friedman's test (Day 2-5) *p* -value	Pooled mean
			Day 1	Day 2	Day 3	Day 4	Day 5		Day 3-5
3-MH (μmol)	Daytime	non-Ex period	75.2 (63.7 to 84.5)	69.8 (59.9 to 86.0)	73.1 (62.2 to 87.2)	66.4 (57.8 to 80.3)	68.6 (62.0 to 74.8)	**0.019**	70.9 (61.1 to 78.6)
		Ex period	84.0 (63.4 to 98.5)	68.5 (58.3 to 87.6)	63.7 (59.1 to 79.7)	64.3 (46.7 to 82.4)	66.7 (63.1 to 76.6)	0.629	64.6 (58.9 to 79.5)
	Nighttime	non-Ex period	70.5 (54.5 to 80.4)	67.9 (57.7 to 71.8)	57.0 (33.2 to 64.9)	55.7 (51.8 to 67.4)	55.8 (38.3 to 68.3)	**0.003**	56.9 * (46.8 to 64.7)
		Ex period	63.4 (50.4 to 72.7)	58.8 # (50.7 to 71.7)	57.2 (49.8 to 64.2)	58.4 (47.3 to 66.2)	58.9 (52.6 to 62.4)	0.323	57.7 (50.0 to 63.0)
	Total	non-Ex period	147.2 (118.2 to 159.6)	135.0 (124.6 to 156.3)	127.4 (110.2 to 145.4)	124.7 (109.5 to 147.7)	124.6 (101.6 to 140.4)	0.060	125.7 * (108.2 to 143.2)
		Ex period	148.6 (114.8 to 166.7)	127.3 (109.1 to 159.3)	120.2 (109.2 to143.6)	121.5 (101.9 to 144.7)	126.1 (115.7 to 139.0)	0.558	122.6 (108.9 to 142.4)
Adrenaline (μg)	Daytime	non-Ex period	3.34 (0.98 to 3.96)	3.39 (1.96 to 6.05)	3.76 (2.02 to 6.26)	3.76 (2.25 to 6.08)	4.02 (2.65 to 5.74)	0.278	3.87 (2.46 to 6.02)
		Ex period	3.48 (2.39 to 6.33)	3.01 (1.74 to 5.15)	5.41 (3.10 to 6.90)	5.43 (2.87 to 11.09)	5.00 (3.63 to 8.79)	**0.003**	5.34 *# (3.61 to 8.83)
	Nighttime	non-Ex period	1.83 (0.97 to 3.13)	2.28 (1.08 to 3.20)	1.91 (1.19 to 2.63)	2.18 (1.55 to 5.25)	2.39 (1.08 to 3.20)	0.218	2.17 (1.43 to 3.48)
		Ex period	1.95 (1.03 to 3.48)	2.51 (0.92 to 3.17)	2.25 (1.33 to 3.72)	2.63 (1.44 to 4.41)	3.11 (2.13 to 5.27)	**0.004**	2.67 (1.69 to 4.41)
	Total	non-Ex period	5.35 (1.95 to 7.09)	5.91 (3.04 to 9.25)	5.97 (3.57 to 8.23)	5.89 (3.80 to 11.33)	6.30 (3.73 to 8.94)	0.082	5.99 (4.08 to 9.50)
		Ex period	5.35 (3.41 to 9.81)	5.67 (2.78 to 8.30)	7.93 (4.55 to 10.34)	7.90 (4.31 to 15.34)	8.18 (6.01 to 14.06)	**0.003**	8.00 *# (5.49 to 13.24)
Noradrenaline (μg)	Daytime	non-Ex period	36.8 (29.6 to 50.5)	42.5 (27.4 to 55.7)	34.9 (27.3 to 51.2)	37.4 (29.4 to 53.2)	37.4 (26.9 to 63.3)	0.558	36.3 (27.9 to 55.6)
		Ex period	39.5 (29.7 to 65.6)	35.7 (27.2 to 54.1)	55.8 (37.2 to 72.5)	61.5 (26.1 to 69.8)	64.9 (34.1 to 71.0)	**0.004**	63.0 *# (32.5 to 67.7)
	Nighttime	non-Ex period	38.1 (22.4 to 55.0)	38.6 (21.9 to 50.2)	30.3 (18.9 to 38.4)	41.7 (19.1 to 52.8)	33.7 (19.0 to 47.6)	**0.001**	34.6 (19.0 to 42.6)
		Ex period	32.5 (22.0 to 46.8)	33.3 (24.8 to 46.9)	36.3 (22.1 to 47.4)	42.0 (22.1 to 55.8)	46.4 (23.3 to 54.6)	**0.011**	43.9 *# (22.5 to 47.0)
	Total	non-Ex period	73.9 (51.9 to 105.5)	81.7 (54.8 to 105.9)	65.8 (46.2 to 89.6)	81.6 (48.5 to 97.2)	70.0 (45.9 to 104.8)	**0.023**	72.2 (46.9 to 97.2)
		Ex period	71.9 (51.8 to 112.3)	68.3 (53.9 to 94.6)	92.4 (59.3 to 119.9)	109.3 (48.1 to 116.7)	111.4 (57.4 to 125.6)	**< 0.001**	107.3 *# (55.0 to 114.6)
Dopamine (μg)	Daytime	non-Ex period	339 (292 to 417)	349 (300 to 526)	355 (303 to 459)	349 (307 to 465)	346 (299 to 454)	0.757	341 (315 to 459)
		Ex period	360 (313 to 454)	338 (288 to 470)	376 (291 to 467)	362 (228 to 447)	360 (292 to 445)	0.450	361 (300 to 451)
	Nighttime	non-Ex period	361 (302 to 516)	343 (304 to 493)	304 (124 to 403)	318 (294 to 454)	317 (232 to 464)	**0.016**	308 * (223 to 440)
		Ex period	334 (285 to 450)	341 (242 to 431)	328 (222 to 389)	315 (244 to 390)	318 (248 to 403)	0.242	320 (250 to 394)

			Adjustment		Fat-free milk intake			Friedman's test (Day 2-5) *p*-value	Pooled mean
			Day 1	Day 2	Day 3	Day 4	Day 5		Day 3-5
Cortisol (μg)	Total	non-Ex period	692 (624 to 933)	689 (614 to 1019)	642 (466 to 862)	662 (603 to 918)	643 (550 to 918)	0.112	649 (551 to 900)
		Ex period	694 (598 to 903)	690 (530 to 901)	704 (548 to 856)	662 (532 to 830)	678 (540 to 849)	0.696	676 (552 to 845)
	Daytime	non-Ex period	23.7 (12.8 to 49.0)	23.8 (13.2 to 55.5)	27.1 (14.7 to 52.7)	28.1 (16.5 to 54.8)	25.8 (22.0 to 41.5)	0.141	27.6 (18.7 to 45.8)
		Ex period	24.7 (16.7 to 49.6)	25.1 (16.4 to 49.6)	30.3 (15.4 to 50.9)	27.7 (12.5 to 71.0)	27.3 (15.6 to 49.0)	0.654	27.4 (17.1 to 56.9)
	Nighttime	non-Ex period	6.0 (3.6 to 13.4)	8.6 (3.7 to 12.0)	7.6 (4.0 to 13.8)	8.6 (5.0 to 13.8)	8.3 (3.1 to 22.1)	0.912	8.4 (4.2 to 15.4)
		Ex period	5.9 (2.4 to 9.7)	5.5 (3.9 to 10.0)	6.2 (5.1 to 11.1)	7.4 (4.4 to 12.9)	6.7 (5.2 to 17.2)	**0.035**	6.7 (5.1 to 13.0)
	Total	non-Ex period	30.1 (17.1 to 55.6)	33.8 (17.9 to 64.8)	36.7 (22.7 to 66.4)	36.4 (27.1 to 65.1)	35.1 (25.1 to 49.3)	0.132	36.0 (25.6 to 57.0)
		Ex period	30.7 (20.6 to 59.4)	29.6 (21.6 to 59.6)	38.6 (20.5 to 59.9)	36.4 (19.9 to 83.9)	34.0 (20.8 to 66.1)	0.323	35.4 (23.0 to 70.0)

N = 8. Variables are given as the medians (minimum value to maximum value).
* p < 0.05 compared to Day 2, # p < 0.05 compared to the non-Ex period.
UN, urea nitrogen; CPR, C-peptide immunoreactivity; 3-MH, 3-metylhistidine.

Fig. 5. Comparison of the degree of change from the second day of the adjustment period to the average value for the three days of the treatment period (Δ) in the urinary C-peptide immunoreactivity (CPR) excretion level. N = 8. Data are shown as the medians (minimum value – maximum value).



4. Discussion

In the present study, we conducted an experiment to examine the effects of BCAA-rich fat-free milk intake, and the effects of exercise in combination with fat-free milk intake on protein-related, muscle synthesis- and breakdown-related, and glucose and lipid metabolism-related parameters based on the hypothesis that the intake of protein containing BCAAs would increase muscle synthesis and suppress muscle breakdown, and that exercise would have beneficial effects on the BCAA intake -induced insulin secretion, and glucose and lipid metabolism. We found that fat-free milk intake increased the serum UN and plasma BCAA levels. During the non-Ex period, the levels of 3-MH, an indicator of muscle protein breakdown, decreased, insulin secretion increased, and the HOMA-IR and AI values increased slightly. In contrast, during the Ex period, insulin secretion, the HOMA-IR and AI values, and the serum TG and RLP-C levels all decreased.

4.1. Protein-related and muscle protein synthesis- and breakdown-related parameters

The increased serum and urinary UN and plasma BCAA levels are thought to reflect the effects of the fat-free milk intake. However, in fat-free milk, the reported amounts per 100 g of edible portion are 2,200 mg of valine, 3,300 mg of leucine, and 1,800 mg of isoleucine,[21] and it is unclear why the isoleucine levels did not also change. The serum UN levels were higher after the Ex period than after the non-Ex period, which may be a result of exercise-induced muscle protein breakdown.[15,22]

For examining muscle protein metabolism, we measured the serum IGF-1 levels as a marker of anabolism,[23,24] and the serum and urinary cortisol levels as markers of catabolism.[25] The serum IGF-1 levels decreased after the Ex period, and were lower after the Ex period than after the non-Ex period. It is known that exercise activates IGF-1,[26] and that the blood concentration of growth hormone, which promotes IGF-1 production, increases transiently during exercise.[27] Therefore, we expected the level to rise in the current study, but the result was the opposite. Since IGF-1 and insulin have similar structures, it may be possible that the IGF-1 levels show changes that are similar to those of insulin. Since the serum CK levels did not increase during the Ex period, it was thought that only a little muscle damage was incurred during the exercise. It was also suggested that cortisol was not affected by the present experimental conditions. A relationship between IGF-1 and cortisol is discussed, so further investigation is required.[28]

3-MH is an indicator of the rate of muscle protein breakdown, and it is released into blood and excreted in urine.[29] The fat-free milk intake-induced decrease in the urinary 3-MH excretion levels was accompanied by decreases in urinary dopamine excretion in the nighttime during the non-Ex period. Similar results were also obtained in our previous research.[30] It has also been reported that the suppression of myofibrillar protein degradation, *i.e.*, a decline in serum 3-MH, was regulated by dietary proteins, and was not synchronized with changes in the serum concentrations of insulin and corticosterone in a rat study.[31] Based on the results of urinary catecholamine excretion, sympathetic action may have been suppressed as a result of the fat-free milk intake. It is possible that the reason no decrease was seen in the 3-MH levels during the Ex period with fat-free

milk intake was because the muscle protein turnover rate was increased.[32,33]

4.2. Glucose and lipid metabolism-related parameters

The urinary CPR excretion levels increased during the non-Ex period with fat-free milk intake, and they decreased during the Ex period with fat-free milk intake. In addition, the serum insulin levels were increased after the non-Ex period, and they were lower after the Ex period than after the non-Ex period. These results are consistent with previous findings regarding protein intake and insulin secretion,[11-13,30] and those regarding exercise and insulin sparing action. Decreases in the plasma and urinary excretion levels of CPR due to exercise have also been reported.[34,35]

The serum TG and RLP-C levels decreased after the Ex period. Furthermore, a high positive correlation was found between the Δ TG and Δ RLP-C. Decreases in the TG level due to exercise have been attributed to an increase in lipoprotein lipase activity,[36] and have been widely reported.[19,37,38] High levels of serum RLP-C constitute a risk factor for atherosclerosis and are associated with insulin resistance.[39,40] Decreases in RLP-C has also been reported.[19,41,42] It is well-known that exercise increases the levels of serum HDL-C.[19,37,38] Although we saw no increase in the HDL-C levels in the present study, significant negative correlations were observed between the Δ HDL-C and Δ TG, and between Δ HDL-C and Δ RLP-C.

As mentioned above, dietary protein promotes insulin secretion; therefore, there remains controversy as to whether the intake of protein, particularly BCAAs, is associated with an increased risk of type 2 diabetes mellitus, based on the findings concerning the BCAA degradation pathway and mammalian target of rapamycin (mTOR).[43-47] A relatively high protein intake during body weight reduction and a moderately high protein diet has positive effects on health in general,[48,49] whereas an excessively high protein intake can have various adverse effects.[50]

There are several limitations in the present study. First, it was difficult to conclusively determine whether the slight increases seen in the HOMA-IR and AI values after the non-exercise period reflected the deterioration of glucose and lipid metabolism due to high protein intake. In fact, the PFC ratio during the treatment period was 17.3:19.3:63.4, so the protein intake was not particularly high, and lipid intake was lower than during the adjustment period. Furthermore, the Δ HOMA-IR showed a significant positive correlation with the Δ RLP-C, and the Δ AI showed a significant positive correlation with the Δ TG and Δ RLP-C, but the serum TG and RLP-C levels did not change after the non-Ex period. The reason for the increase in the serum LDL-C levels after both the non-Ex and Ex periods is also unclear. Second, the present study was a short observational study that examined treatments for only 3 days. Further studies that include additional examinations of body protein anabolic and catabolic action over a longer intervention period are required to determine suitable dietary and exercise therapies for treating metabolic syndrome and sarcopenia. Third, it may be necessary to also consider possible differences between sexes. Taken together, the results of the present study indicated that exercise attenuated the insulinotropic effects of protein intake.

Therefore, exercise may increase muscle mass and strength in an insulin-independent manner, and such insulin-sparing action is known to have beneficial effects against metabolic syndrome.

5. Conclusion

In the present study, we found that exercise attenuated the insulinotropic effects of protein, particularly BCAAs intake, and had beneficial effects on glucose and lipid metabolism. Thus, higher protein intake in conjunction with exercise seems to be a reasonable recommendation for preventing and/or improving both metabolic syndrome and sarcopenia.

Conflict of Interest Statement:

The authors have no conflicts of interest to declare.

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Abbreviations

AI, atherosclerosis index; BCAA, branched chain amino acid; BG, blood glucose; CK, creatine kinase; CPR, C-peptide immunoreactivity; FFA, free fatty acid; HDL-C, high density lipoprotein-cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance; IGF-1, insulin-like growth factor 1; IRI, immunoreactive insulin; LDL-C, low density lipoprotein-cholesterol; 3-MH, 3-methylhistidine; PFC, protein:fat:carbohydrate; RLP-C, remnant lipoprotein-cholesterol; TC, total cholesterol; TG, triglyceride; UN, urea nitrogen

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