



RESEARCH ARTICLE

# The Insulin-Sparing Effects of Exercise are Attenuated by a High-Protein Diet While Maintaining the Effects of Exercise on Glucose and Lipid Metabolism

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## ABSTRACT

**Background:** Metabolic syndrome and sarcopenia are physical conditions that are addressed differently through nutrition and exercise. Proteins, particularly branched-chain amino acids (BCAAs), exert insulinotropic effects, whereas exercise reduces insulin secretion. It is thus necessary to understand the effects of protein intake and exercise alone and in combination when considering suitable dietary and exercise therapies for treating metabolic syndrome and sarcopenia.

**Methods:** Seven healthy young-adult male volunteers participated in a crossover trial consisting of two 6-day experiments. Days 1-3 comprised a body-weight-maintained adjustment period during which the participants consumed a control diet (energy, 2,500 kcal; protein, 61.3 g for an individual weighing 60 kg). Days 4-6 comprised the treatment period during which the participants expended 402 kcal of additional energy by riding a bicycle with an ergometer and consumed an additional 402 kcal, either as BCAA-rich fat-free milk (high-protein diet + Exercise (HP + Ex) period) in one experiment or carbohydrates and lipids only (non-high-protein diet + Exercise (non-HP + Ex) period) in another experiment. Total 24-h urine samples were collected daily throughout the study. Fasting blood samples were collected early in the morning before and after the treatment period.

**Results:** Plasma valine, BCAA, and serum urea nitrogen levels were significantly elevated only after the HP + Ex period. Serum insulin-like growth factor 1 (IGF-1) levels were significantly decreased after both the non-HP + Ex and HP + Ex periods, but there was no difference in the degree of change ( $\Delta$ ) in serum IGF-1 level ( $\Delta$  serum IGF-1) (amount of decrease in IGF-1) between the two periods. Urinary C-peptide immunoreactivity excretion levels decreased significantly during the non-HP + Ex period but were unchanged during the HP + Ex period. Serum triglyceride and remnant lipoprotein-cholesterol levels were significantly lower after both the non-HP + Ex and HP + Ex periods but did not differ between the two periods. Furthermore, there were no significant differences between the two periods in  $\Delta$  serum triglyceride and  $\Delta$  serum remnant lipoprotein-cholesterol (amount of decrease). Urinary noradrenaline and dopamine excretion levels were significantly higher during the HP + Ex period than during the non-HP + Ex period, and the  $\Delta$  urinary noradrenaline and  $\Delta$  urinary dopamine (amount of increase) were also significantly greater during the HP + Ex period than during the non-HP + Ex period.

**Conclusions:** A high-protein diet during exercise attenuates the insulin-sparing effects but does not affect the effects on glucose and lipid metabolism, although its effects on body protein synthesis are unclear. The combination of a high-protein diet and exercise is suggested to be an effective regimen for sarcopenic obesity.

## Abbreviations

AI, atherosclerosis index  
BCAA, branched chain amino acid  
BG, blood glucose  
CK, creatine kinase  
CPR, C-peptide immunoreactivity  
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  
RLP-C, remnant lipoprotein-cholesterol  
TC, total cholesterol  
TG, triglyceride  
UN, urea nitrogen

## 1. Introduction

Metabolic syndrome and sarcopenia are serious medical conditions of urgent public health concern, as their worldwide prevalence has increased with expansion of lifestyle-related diseases and aging population.<sup>1,2</sup> As the approaches for treating metabolic syndrome and sarcopenia differ, addressing sarcopenic obesity is challenging. Both nutrition and exercise are important factors that must be considered in addressing metabolic syndrome and sarcopenia. To address metabolic syndrome, it is necessary to restrict energy intake and reduce body weight while ensuring adequate nutrient intake.<sup>3</sup> Obesity, hypertension, and impaired glucose and lipid metabolism can be alleviated through aerobic exercise, which directly promotes the expenditure of glucose and free fatty acids.<sup>4-6</sup> In addressing sarcopenia, by contrast, it is necessary to ensure adequate energy intake and supplement if necessary, in addition to ensuring an adequate supply of nutrients, such as protein and vitamin D.<sup>7</sup> Regarding exercise to prevent or improve sarcopenia, resistance exercises, which involve a relatively large mechanical load, are effective for increasing muscle mass and strength and indirectly promote glucose and lipid metabolism.<sup>8</sup>

Insulin lowers the blood glucose (BG) level and stimulates protein anabolism.<sup>9</sup> From the viewpoint of metabolic syndrome, it is desirable to avoid excessive insulin secretion. Proteins, particularly branched-chain amino acids (BCAAs), are utilized for energy,<sup>10</sup> body protein maintenance and anabolism;<sup>11, 12</sup> however, they also exert insulinotropic effects.<sup>13-15</sup> Exercise also promotes body protein synthesis while reducing insulin secretion.<sup>16,17</sup> Thus, although both BCAA intake and exercise affect body protein anabolism, they exert opposite effects on insulin secretion. When considering dietary and exercise therapies for treating metabolic syndrome and sarcopenia, therefore, it is necessary to investigate the effects of protein intake and exercise alone and in combination. In addition, it is important to consider the effects of increased insulin secretion by protein intake on glucose and lipid metabolism.

We previously focused our research on body weight loss as it relates to glucose and lipid metabolism, nutrient balance, and indicators related to muscle mass. That is,

reducing body weight via exercise had more beneficial effects on lipid metabolism than a dietary approach. The improvement in insulin resistance was accompanied by decreases in serum triglyceride (TG), remnant lipoprotein-cholesterol (RLP-C), and apolipoprotein B48 levels.<sup>18</sup> In addition, nitrogen, sodium, and potassium balance in our study was significantly more positive during the exercise regimen than during the diet regimen. Urinary excretion levels of creatinine, 3-methylhistidine (3-MH), aldosterone, catecholamines, and the 3-MH/creatinine ratio were significantly increased only during the exercise regimen.<sup>19</sup>

In another previous study,<sup>20</sup> we examined the effects of consuming BCAA-rich fat-free milk in combination with exercise or without exercise on protein-related parameters, including plasma BCAA level, muscle protein synthesis— and breakdown—related parameters, and glucose and lipid metabolism—related parameters, including serum insulin level, urinary C-peptide immunoreactivity (CPR) excretion level, homeostatic model assessment for insulin resistance (HOMA-IR), and atherosclerosis index (AI). Plasma BCAA levels were significantly elevated after both the non-exercise and exercise periods, whereas serum insulin levels were significantly elevated only after the non-exercise period. Urinary CPR excretion levels increased significantly during the non-exercise period but showed no significant changes during the exercise period. Serum TG and RLP-C levels were significantly reduced after the exercise period. Thus, exercise attenuated the insulinotropic effects of protein intake and exerted beneficial effects on glucose and lipid metabolism. However, serum insulin-like growth factor 1 (IGF-1) levels remained unchanged after the non-exercise period but were lower after the exercise period.

Based on these previous results,<sup>20</sup> in the present study, we examined the effect of exercise in combination with BCAA-rich fat-free milk consumption and without consumption, under the hypothesis that serum insulin and urinary CPR excretion levels would increase during exercise in subjects consuming fat-free milk compared with those not consuming fat-free milk. We also examined potential differences in muscle protein synthesis— and breakdown—related parameters as well as glucose and lipid metabolism—related parameters between with/without consumption of BCAA-rich fat-free milk.

## 2. Materials and Methods

### 2.1 SUBJECTS

Seven healthy young-adult male volunteers (age,  $20 \pm 1$  years [mean  $\pm$  standard deviation]; body height,  $175 \pm 7$  cm; body weight,  $64.7 \pm 8.2$  kg) participated in the metabolic experiment. A subject briefing session was held prior to the experiment in order to explain the methods of the study, including the 12-day restriction of usual life activities, dietary control, exercise load, and blood and urine sample collections. Individuals with allergies to fat-free milk were excluded.

Consequently, seven subjects were enrolled, which was the small number needed for the statistical analyses. G\*Power 3.1 software was used for power analyses,<sup>21, 22</sup>

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, the power for detecting differences in serum TG level after exercise was 85%.<sup>18</sup> In another previous study with a sample size of 8, the power for detecting post-exercise differences in serum TG and RLP-C levels was 98% and >99%, respectively. Furthermore, the power was >99% for detecting differences in urinary CPR excretion with and without exercise.<sup>20</sup> All subjects provided written informed consent to participate in all procedures associated with the study, which adhered to the tenets of the Declaration of Helsinki.

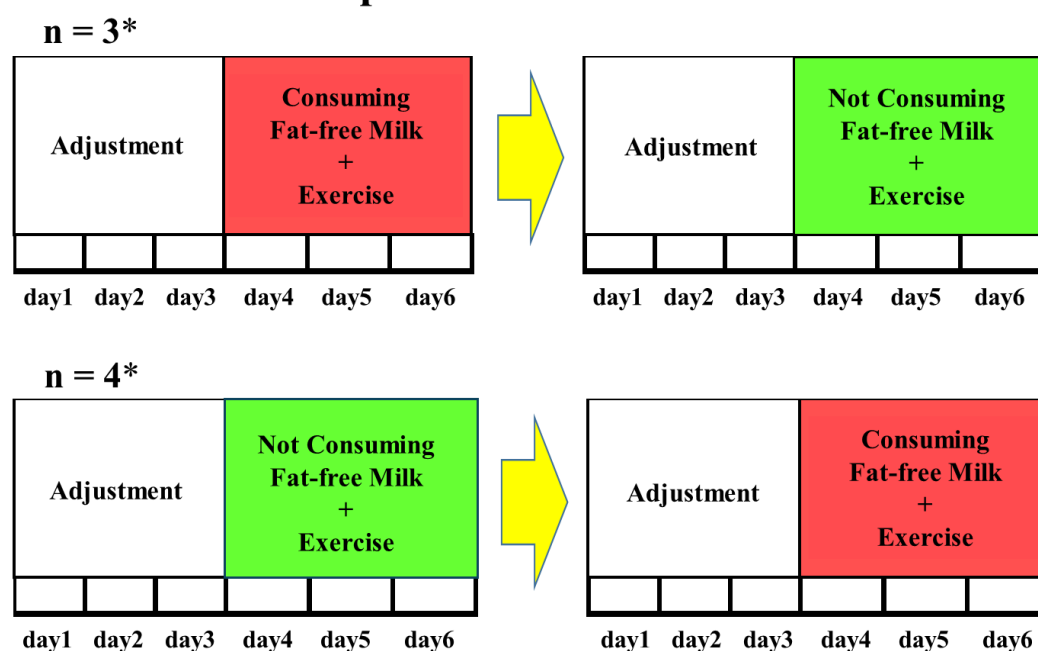
## 2.2 EXPERIMENTAL PROCEDURES

The Research Ethics Committee of Kanto Gakuin University approved the study protocols (approval number: 2016-1-1).

### 2.2.1 Experimental protocol

The study comprised two 6-day experiments (12 days in total; **Fig. 1**). All 7 subjects participated in both experiments. Each experiment consisted of a 3-day treatment period preceded by a 3-day body-weight-maintained adjustment period (6 days in total). During the adjustment period, the participants consumed control diets. During the treatment period, the participants exercised, and the additional energy provided to the control diet during the adjustment period was consumed as either BCAA-rich fat-free milk (high protein diet) in one experiment or as carbohydrates and lipids only (non-high protein diet) in the other experiment. After the end of the first 6-day experiment, a washout period of >7 days was initiated, during which the subjects consumed an *ad libitum* diet before the second 6-day experiment started. The two experiments were performed as a crossover trial, (i.e., high-protein diet with exercise [HP + Ex] was followed by non-high protein diet with exercise [non-HP + Ex]) for three participants, and the order was reversed for the remaining four participants.

## Experimental schedule



\* Seven adult male volunteers participated in a crossover trial, i.e., two experiments over 6 days.

**Fig. 1.** Experimental protocol. After the end of the first 6-day experiment, a >7-day wash-out period was implemented, during which the subjects had access to an *ad libitum* diet before the second 6-day experiment started.

For each experiment, the subjects 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 in the laboratory during the day and in the metabolic experiment building at night.

During the adjustment period from day 1 to day 3, the subjects woke up at 06:30, collected the final urine sample for the previous day at 07:00, then began collecting all urine for the next 24 h from that point. Body weight and body composition were measured using an InBody 720 body composition analyzer (InBody Japan Inc., Tokyo, Japan). Breakfast was served between 08:30

and 09:00, lunch was served between 12:30 and 13:00, and dinner was served between 18:00 and 18:30. Other than taking a shower between 19:30 and 21:30, and going to bed at 22:00, they were free to spend their time in the laboratory and metabolic experiment building, with the exception of eating or engaging in additional physical activity.

The treatment (non-HP + Ex and HP + Ex) periods lasted from day 4 to day 6. During both periods, 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 15:00 in the afternoon. In addition to the diet during

the adjustment period, the amount of additional energy expended during exercise was added.

Throughout the experiment, the room was maintained at a dry bulb temperature of 25-26°C and wet bulb temperature of 22-23°C.

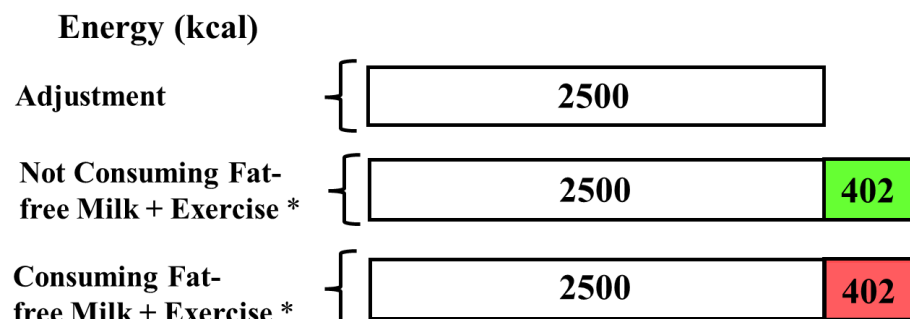
### 2.2.2 Experimental diet

The experimental diet during the adjustment period, *i.e.*, the control diet consisted of the following basically homogeneous foods: crackers, margarine, strawberry jam, mashed potatoes, salt and pepper, vegetable juice, biscuits, spaghetti, meat sauce, vegetable oil, vitamin mixture beverages, milled rice, soy protein processed food, tomato ketchup, miso, *Komachi-fu* (a gluten product), and seaweed *tsukudani*. During the body-weight-maintained adjustment period, the experimental diet was set to meet the recommended dietary allowance or adequate intake as specified in the Dietary Reference Intakes for Japanese.<sup>23</sup> The amount of food consumed each day was adjusted according to the body weight of each participant. The calculated values per day for an individual weighing 60 kg based on the Standard Tables of Food Composition in Japan<sup>24</sup> were as follows: energy, 2,500 kcal; protein, 61.3 g; lipid, 73.0 g; carbohydrate, 387.9 g. The BCAA content was 6.4 g (isoleucine, 1.5 g; leucine, 3.0 g; and valine, 1.9 g). Actual energy intake

during the adjustment period was  $2643 \pm 224$  kcal/day.

The additional energy provided to the control diet was consumed during the treatment periods. The amount of additional energy was adjusted according to the energy intake level per day during the adjustment period. During the HP + Ex period for an individual weighing 60 kg, 402 kcal of fat-free milk was consumed divided into each of three meals per day, the volume of which was determined by calculating the equivalent amount of energy (134 kcal) in 200 ml of whole milk per meal, which was considered to be a normal amount of milk consumed in daily life. Thus, the intake levels during the HP + Ex period were as follows: energy, 2,902 kcal; protein, 102.2 g; lipid, 73.7 g; carbohydrate, 446.0 g; BCAAs, 14.5 g (isoleucine, 3.5 g; leucine, 6.7 g; and valine, 4.3 g). During the non-HP + Ex period, additional energy was consumed as carbohydrates and lipids only, so that the fat energy ratio remained the same as during the adjustment period, which were supplied through cornstarch, white superior soft sugar, shortening and carbonated drink.<sup>25, 26</sup> Therefore, the intake levels during the non-HP + Ex period for an individual weighing 60 kg were as follows: energy, 2,902 kcal; protein, 61.3 g; lipid, 84.6 g; carbohydrate, 461.5 g. Actual additional energy intake during both the non-HP + Ex and HP + Ex periods was  $425 \pm 36$  kcal/day (**Fig. 2**).

## Energy and composition of experimental diet (in case of 60 kg of body weight)



	Adjustment	Not Consuming Fat-free Milk + Exercise	Consuming Fat-free Milk + Exercise
Protein (g)	61.3	61.3	102.2
Lipid (g)	73.0	84.6	73.7
Carbohydrate (g)	387.9	461.5	446.0

\* 402 kcal/day of additional energy was expended during Exercise period.

**Fig. 2.** Energy and composition of experimental diet.

### 2.2.3 Exercise procedures

Before the start of the study, an exercise tolerance test was performed, which involved gradually increasing the work rates on a bicycle with an ergometer. Heart rate was recorded via telemetry (DS-3400; Fukuda Denshi Co., Ltd., Tokyo, Japan). Samples of expired gas were collected before and at steady state during exercise using a Douglas bag, and gas volume was measured using a dry gas meter (DC-5A; Shinagawa Corp., Tokyo,

Japan). 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 additional energy expenditure parameters were calculated based on oxygen intake and the respiratory exchange ratio, work rate (kilopounds [kp] of the bicycle ergometer), and heart rate for each subject. During the treatment periods, half of the additional energy was expended at a

moderate intensity in the morning, and the other half was expended at a vigorous intensity in the afternoon. Moderate-intensity exercise was performed at about 50% of the maximal oxygen intake, and vigorous-intensity exercise was performed by increasing the kp level 1.6 to 2 times greater than that used for moderate-intensity exercise. Exercise duration was  $47.3 \pm 5.0$  min in the morning and  $25.9 \pm 4.3$  min in the afternoon.

### 2.3. SAMPLE COLLECTION AND MEASUREMENTS

Fasting blood samples were collected early in the morning before the first day of each treatment period (the 3rd day of each 6-day experiment) and at the end of each treatment period (the morning after day 6 of each 6-day experiment). Total urine samples were collected on a 24-h basis (07:00 to 07:00 the next day) daily throughout each experiment.

Blood and urine samples were sent to the following laboratories: BML Inc. (Tokyo, Japan) for measurements of BG, serum urea nitrogen (UN), creatine kinase (CK), immunoreactive insulin (IRI), TG, total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), and high-density lipoprotein-cholesterol (HDL-C) levels; SRL Inc. (Tokyo, Japan) for determination of levels of plasma amino acids (valine, leucine, isoleucine, and lysine), serum IGF-1, free-testosterone, cortisol, RLP-C, urinary CPR, 3-MH, and cortisol; LSI Medience Corp. (Tokyo, Japan) for measurements of the levels of urinary creatinine, UN, and three types of catecholamines (adrenaline, noradrenaline, and dopamine); and Material Research Center Co., Ltd. (Tokyo, Japan) for measurement of serum myostatin levels.

HOMA-IR was calculated according to the formula (fasting blood glucose [mg/dl]  $\times$  fasting insulin [ $\mu$ U/ml]/405). AI was calculated according to the formula  $[(TC - HDL-C) / HDL-C]$ .

### 2.4. STATISTICAL ANALYSES

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

## 3. Results

### 3.1. BODY WEIGHT AND COMPOSITION

The changes in the body weight and composition are shown in **Table 1**. Although body weight increased slightly during the non-HP + Ex period, body fat percentage, body fat mass, and skeletal muscle mass remained almost unchanged during both the non-HP + Ex and HP + Ex periods.

### 3.2. BLOOD METABOLIC PARAMETERS

The changes in blood levels of protein-related parameters (UN, BCAAs, and lysine), muscle synthesis- and breakdown-related parameters (IGF-1, free-testosterone, myostatin, CK, and cortisol), and glucose and lipid metabolism-related parameters are shown in **Table 2**.

Table 1. Changes in the body weight and composition.

		Day 1	Adjustment Day 2	Day 3	Day 4	Treatment Day 5	Day 6	After	Friedman's test (Day 4-After) <i>p</i> -value
Body weight (kg)	non-HP + Ex period	64.3 (53.0 to 78.9)	64.6 (52.7 to 79.0)	64.1 (52.6 to 78.3)	64.4 (52.4 to 78.2)	64.5 (52.4 to 78.2)	64.5 (52.5 to 78.6)	64.7 (52.5 to 79.2)	<b>0.002</b>
	HP + Ex period	65.8 (53.2 to 79.0)	65.4 (52.7 to 79.0)	65.1 (52.7 to 79.0)	64.6 (52.6 to 78.8)	65.0 (52.5 to 78.9)	64.8 (52.2 to 79.0)	65.0 (52.2 to 79.1)	0.309
Body fat percentage (%)	non-HP + Ex period	12.1 (8.9 to 27.3)	12.3 (9.2 to 27.5)	12.0 (10.1 to 28.7)	12.0 (10.3 to 28.2)	12.2 (9.7 to 28.2)	11.4 (10.5 to 28.2)	12.0 (10.2 to 27.6)	0.932
	HP + Ex period	11.9 (9.7 to 28.8)	12.4 (10.2 to 28.1)	12.7 (10.6 to 28.8)	12.5 (10.4 to 28.0)	12.9 (10.3 to 28.4)	12.9 (10.2 to 28.6)	11.3 (10.5 to 28.6)	0.263
Body fat mass (kg)	non-HP + Ex period	7.9 (4.7 to 21.5)	8.4 (4.8 to 21.7)	8.4 (5.3 to 22.4)	8.3 (5.4 to 22.1)	8.5 (5.1 to 22.1)	7.8 (5.5 to 22.2)	8.3 (5.7 to 21.9)	0.835
	HP + Ex period	8.0 (5.2 to 22.7)	8.2 (5.5 to 22.2)	8.4 (5.6 to 22.8)	8.1 (5.5 to 22.0)	8.5 (5.6 to 22.4)	8.5 (5.5 to 22.6)	7.6 (5.5 to 22.6)	0.287
Skeletal muscle mass (kg)	non-HP + Ex period	32.2 (27.3 to 35.8)	32.1 (27.0 to 34.8)	31.7 (26.8 to 34.8)	31.9 (26.6 to 34.7)	31.8 (26.9 to 34.7)	32.0 (26.6 to 35.3)	32.5 (26.4 to 35.0)	0.851
	HP + Ex period	31.9 (27.2 to 35.1)	32.3 (26.7 to 35.3)	32.0 (26.8 to 35.1)	32.1 (26.8 to 35.2)	32.1 (26.7 to 35.3)	32.0 (26.5 to 35.6)	32.0 (26.5 to 35.2)	0.564

n = 7. Variables are given as the medians (minimum value to maximum value).



Table 2. Changes in the blood metabolic parameters.

Variable	non-HP + Ex period			HP + Ex period			$\Delta^1$ vs $\Delta^2$ <i>p</i> - value
	Before	After	$\Delta^1$	Before	After	$\Delta^2$	
UN (mg/dl)	11.4 (8.5 to 16.0)	11.0 (9.2 to 15.6)	+0.1 (−2.1 to +1.6)	10.7 (8.4 to 14.3)	14.6 *# (12.9 to 18.6)	+4.4 (+0.3 to +5.8)	<b>0.031</b>
Valine (nmol/ml)	254.3 (213.1 to 261.0)	244.7 (189.4 to 249.4)	−12.9 (−46.4 to +15.1)	236.0 (209.4 to 268.0)	259.1 *# (229.4 to 290.9)	+18.2 (+11.5 to +81.5)	<b>0.031</b>
Leucine (nmol/ml)	151.4 (116.0 to 163.3)	140.5 (108.0 to 161.1)	−8.0 (−16.9 to +10.1)	150.8 (129.9 to 168.8)	152.5 (133.5 to 173.9)	+9.5 (−22.4 to +23.1)	0.219
Isoleucine (nmol/ml)	69.4 (59.1 to 94.2)	73.5 (53.6 to 82.8)	−4.5 (−13.7 to +13.4)	69.7 (54.1 to 88.5)	73.6 (57.1 to 87.0)	+3.0 (−14.1 to +17.3)	0.469
BCAAs (nmol/ml)	464.7 (388.2 to 518.5)	465.7 (351.0 to 484.0)	−26.4 (−69.3 to +19.3)	449.6 (395.8 to 524.5)	490.3 *# (420.0 to 551.8)	+29.3 (−20.7 to +121.9)	0.078
Lysine (nmol/ml)	186.4 (164.9 to 217.5)	170.8 * (145.6 to 211.3)	−6.2 (−40.8 to +0.2)	173.0 (148.6 to 203.0)	189.8 # (166.6 to 212.5)	+17.2 (−12.1 to +53.3)	<b>0.031</b>
IGF-1 (ng/ml)	234 (173 to 298)	209 * (152 to 273)	−21 (−52 to +4)	250 # (201 to 303)	223 *# (205 to 278)	−16 (−31 to +4)	1.000
Free-testosterone (pg/ml)	16.7 (9.6 to 21.3)	17.5 (12.6 to 19.9)	+0.8 (−3.8 to +3.0)	15.6 (12.2 to 20.9)	15.9 (11.9 to 20.3)	+0.2 (−2.8 to +1.9)	0.453
Myostatin (ng/ml)	75.5 (61.0 to 81.2)	68.3 (58.0 to 96.4)	−5.3 (−15.8 to +15.2)	65.9 (58.8 to 78.8)	74.8 (57.7 to 82.1)	+6.8 (−3.5 to +15.2)	0.406
CK (U/l)	123 (89 to 183)	155 (61 to 180)	+32 (−52 to +44)	105 (79 to 254)	128 (90 to 198)	+11 (−70 to +61)	0.688
Cortisol (μg/dl)	10.60 (5.19 to 13.80)	10.20 (7.95 to 14.30)	+0.50 (−2.70 to +4.39)	11.30 (6.22 to 13.70)	9.96 (7.83 to 14.30)	+0.10 (−2.53 to +3.74)	1.000
BG (mg/dl)	80 (75 to 87)	81 (77 to 85)	+2 (−10 to +6)	80 (75 to 86)	80 (78 to 86)	+1 (−3 to +3)	0.875
IRI (μU/ml)	5.6 (1.5 to 7.9)	4.5 (2.7 to 9.1)	+0.1 (−3.7 to +3.5)	6.2 (1.5 to 7.4)	5.2 (4.2 to 10.8)	−0.2 (−2.0 to +3.7)	0.578
TG (mg/dl)	111 (57 to 201)	72 * (40 to 162)	−28 (−88 to −4)	120 (69 to 159)	87 * (39 to 103)	−49 (−68 to −28)	0.313
RLP-C (mg/dl)	4.9 (2.5 to 9.0)	3.5 * (1.9 to 8.6)	−1.4 (−4.0 to −0.4)	5.9 (2.8 to 8.5)	3.6 * (1.6 to 5.2)	−2.8 (−4.2 to −1.2)	0.109
TC (mg/dl)	162 (144 to 188)	165 (133 to 189)	−5 (−28 to +21)	188 (135 to 218)	185 # (135 to 194)	−7 (−27 to +4)	0.688
LDL-C (mg/dl)	86 (68 to 123)	91 (64 to 121)	−1 (−16 to +32)	98 (66 to 157)	96 # (71 to 140)	−2 (−17 to +12)	0.688
HDL-C (mg/dl)	60 (42 to 71)	58 (41 to 72)	−1 (−8 to +5)	60 (45 to 74)	61 (44 to 75)	+1 (−1 to +4)	0.188
HOMA-IR	1.12 (0.28 to 1.64)	0.88 (0.51 to 1.86)	+0.11 (−0.89 to +0.74)	1.21 (0.28 to 1.46)	1.02 (0.83 to 2.21)	−0.09 (−0.38 to +0.75)	0.688
AI	1.67 (1.27 to 3.48)	1.57 (1.25 to 3.46)	−0.01 (−0.27 to +0.63)	1.79 # (1.41 to 3.84)	1.64 * (1.27 to 3.34)	−0.13 (−0.50 to 0.00)	0.109

n = 7. Variables are given as the medians (minimum value to maximum value).

$\Delta^1$ , degree of change during non-HP + Ex period;  $\Delta^2$ , degree of change during HP + Ex period.

\*  $p < 0.05$  compared to before non-HP + Ex period or HP + Ex period, #  $p < 0.05$  compared to after non-HP + 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;

TG, triglyceride; RLP-C, remnant lipoprotein-cholesterol; TC, total cholesterol; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol;

HOMA-IR, homeostatic model assessment for insulin resistance; AI, atherosclerosis index

Serum UN levels were unchanged after the non-HP + Ex period but significantly increased after the HP + Ex period. The levels were significantly higher after the HP + Ex period than after the non-HP + Ex period, and the degree of change ( $\Delta$ ) in serum UN level ( $\Delta$  serum UN; amount of increase) was significantly greater after the HP + Ex period than after the non-HP + Ex period. The results for plasma valine and BCAA levels were the same as those for serum UN. By contrast, no significant changes in plasma leucine and isoleucine levels were observed after both the non-HP + Ex and HP + Ex periods. Plasma lysine levels were significantly higher after the HP + Ex period than after the non-HP + Ex period, and the  $\Delta$  plasma lysine (amount of increase) was significantly

greater after the HP + Ex period than after the non-HP + Ex period.

Serum IGF-1 levels were significantly decreased after both the non-HP + Ex and HP + Ex periods. The levels were significantly higher after the HP + Ex period than after the non-HP + Ex period, but there was no significant difference in  $\Delta$  serum IGF-1 (amount of decrease). No significant changes were observed in serum free-testosterone, myostatin, CK and cortisol levels, and no significant differences were observed between the two periods.

BG levels remained fairly constant after both the non-HP

+ Ex and HP + Ex periods. Serum IRI levels also showed no significant changes. Serum TG and RLP-C levels were significantly decreased after both the non-HP + Ex and HP + Ex periods, but there were no significant differences after the two periods. Furthermore, there were no significant differences between the two periods in the  $\Delta$  serum TG and  $\Delta$  serum RLP-C (amount of decrease). Serum TC and LDL-C levels were significantly higher after the HP + Ex period than after the non-HP + Ex period, but there were no significant differences between the two periods in terms of  $\Delta$  serum TC and  $\Delta$  serum LDL-C. No significant changes in serum HDL-C levels were observed after the non-HP + Ex and HP + Ex periods. HOMA-IR

values, were similar to the serum IRI levels, showing no significant changes. AI values were significantly decreased after the HP + Ex period, but there was no significant difference between the two periods in terms of  $\Delta$  AI.

### 3.3. URINARY METABOLIC PARAMETERS

The daily changes and pooled mean data for the excretion levels of urinary creatinine, UN, 3-MH, cortisol, CPR, and catecholamines (adrenaline, noradrenaline, dopamine) are shown in **Table 3**. The  $\Delta$  urinary UN,  $\Delta$  urinary CPR,  $\Delta$  urinary noradrenaline, and  $\Delta$  urinary dopamine values are shown in **Figure 3**.

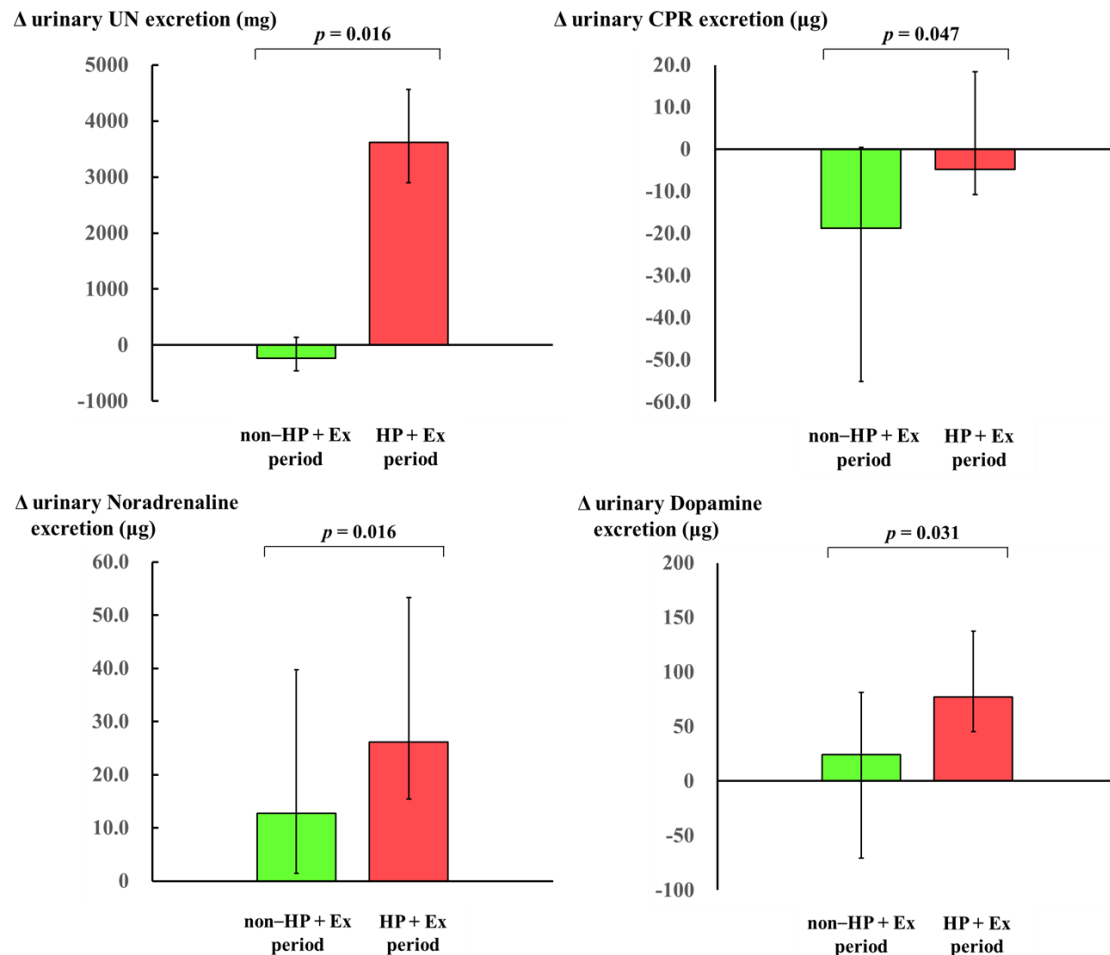
Table 3. Changes in the excretion levels of urinary metabolic parameters.

		Day 1	Adjustment Day 2	Day 3	Day 4	Treatment Day 5	Day 6	Friedman's test (Day 3-6) <i>p</i> -value	Pooled mean Day 4-6
Creatinine (mg)	non-HP + Ex period	1568 (1356 to 1816)	1706 (1174 to 1875)	1625 (1348 to 1860)	1675 (1247 to 1911)	1675 (1411 to 1942)	1691 (1003 to 1827)	0.652	1685 (1330 to 1885)
	HP + Ex period	1719 (1367 to 1891)	1690 (1384 to 1946)	1720 (1386 to 1879)	1647 (1406 to 1931)	1654 (1211 to 1904)	1717 (1414 to 1939)	0.235	1651 (1348 to 1923)
UN (mg)	non-HP + Ex period	8763 (7439 to 10306)	8168 (6164 to 9645)	8300 (6889 to 9032)	8146 (6211 to 9286)	8228 (7221 to 8418)	8096 (4737 to 8758)	0.906	8062 * (6542 to 8763)
	HP + Ex period	8382 (5677 to 9481)	8390 (6190 to 9171)	8232 (6723 to 8913)	10134 (9612 to 11790)	12630 (8780 to 13863)	13348 (11756 to 14449)	< 0.001	12086 *# (10338 to 13283)
3-MH ( $\mu$ mol)	non-HP + Ex period	268.6 (207.2 to 339.7)	266.3 (175.0 to 288.6)	252.5 (201.5 to 286.5)	263.7 (186.8 to 304.3)	258.4 (209.6 to 322.2)	257.0 (155.8 to 312.2)	0.524	257.4 (201.9 to 312.9)
	HP + Ex period	285.1 (193.7 to 312.4)	268.7 (192.3 to 276.3)	262.4 (195.1 to 279.9)	264.5 (213.5 to 284.2)	255.6 (173.4 to 306.8)	252.8 (210.0 to 318.5)	0.757	258.0 (202.2 to 303.2)
Cortisol ( $\mu$ g)	non-HP + Ex period	56.4 (47.2 to 90.0)	47.3 (31.6 to 119.6)	58.5 (43.7 to 118.7)	51.2 (30.8 to 96.8)	62.7 (37.0 to 87.5)	49.7 (33.3 to 98.2)	0.366	54.2 (33.7 to 94.2)
	HP + Ex period	55.0 (44.6 to 74.7)	51.6 (45.6 to 77.3)	65.1 (42.7 to 74.2)	65.7 (47.0 to 100.3)	58.5 (40.3 to 87.3)	64.0 (45.9 to 96.2)	0.017	58.8 (44.4 to 94.6)
CPR ( $\mu$ g)	non-HP + Ex period	75.2 (35.6 to 123.0)	71.3 (36.7 to 132.7)	77.2 (36.2 to 159.2)	53.9 (40.4 to 120.8)	54.5 (30.9 to 93.8)	38.5 (34.9 to 97.4)	< 0.001	53.6 * (35.9 to 104.0)
	HP + Ex period	58.6 (42.8 to 106.8)	68.5 (47.1 to 115.2)	64.3 (56.1 to 128.9)	80.5 (51.1 to 120.1)	69.5 (44.9 to 120.8)	97.8 (47.8 to 113.4)	0.652	82.6 # (47.9 to 118.1)
Adrenaline ( $\mu$ g)	non-HP + Ex period	13.68 (5.70 to 20.70)	11.32 (4.64 to 15.75)	10.20 (7.01 to 22.03)	14.14 (6.18 to 25.39)	12.80 (6.32 to 16.37)	10.77 (6.74 to 18.05)	0.224	13.99 (6.41 to 19.94)
	HP + Ex period	12.34 (4.18 to 18.72)	9.85 (4.78 to 17.80)	11.97 (5.97 to 29.64)	15.46 (7.58 to 26.43)	15.40 (7.09 to 20.33)	14.92 (7.97 to 22.37)	0.122	16.42 # (7.55 to 23.04)
Noradrenaline ( $\mu$ g)	non-HP + Ex period	105.5 (86.7 to 138.0)	93.1 (62.9 to 112.8)	103.0 (68.4 to 126.0)	127.2 (67.8 to 155.2)	113.2 (69.0 to 156.1)	91.9 (74.9 to 143.3)	0.030	115.7 * (70.6 to 151.5)
	HP + Ex period	114.2 (55.2 to 144.0)	107.0 (59.0 to 115.6)	104.0 (60.0 to 113.7)	132.4 (79.5 to 164.8)	130.1 (86.9 to 165.0)	122.9 (85.6 to 176.4)	0.001	128.5 *# (84.8 to 161.9)
Dopamine ( $\mu$ g)	non-HP + Ex period	888 (801 to 1034)	803 (636 to 1018)	817 (692 to 1025)	850 (726 to 1012)	884 (675 to 1034)	813 (539 to 992)	0.289	829 (716 to 1013)
	HP + Ex period	931 (788 to 1162)	854 (780 to 1089)	803 (788 to 1059)	981 (801 to 1200)	877 (775 to 1170)	942 (806 to 1143)	0.012	904 *# (846 to 1171)

n = 7. Variables are given as the medians (minimum value to maximum value).

\*  $p < 0.05$  compared to Day 3, #  $p < 0.05$  compared to non-HP + Ex period.

UN, urea nitrogen; 3-MH, 3-methylhistidine; CPR, C-peptide immunoreactivity.



**Fig. 3.** Comparison of the degree of change from the 3rd day of the adjustment period to the average value for the 3 days of the treatment period ( $\Delta$ ).  $n = 7$ . Data are shown as the median (minimum value – maximum value).

Urinary creatinine excretion levels remained fairly constant during both the non-HP + Ex and HP + Ex periods, with no significant difference between the two periods. Urinary UN excretion levels remained unchanged during the non-HP + Ex period but increased significantly during the HP + Ex period and were significantly higher during the HP + Ex period than during the non-HP + Ex period. Furthermore, the  $\Delta$  urinary UN (amount of increase) was significantly greater during the HP + Ex period than during the non-HP + Ex period. Urinary 3-MH excretion levels remained unchanged during both the non-HP + Ex and HP + Ex periods, with no difference between the two periods in  $\Delta$  urinary 3-MH. Urinary cortisol excretion levels remained almost unchanged during both periods. Urinary CPR excretion levels decreased significantly during the non-HP + Ex period but remained unchanged during the HP + Ex period, and the excretion levels were significantly lower during the non-HP + Ex period than during the HP + Ex period. The  $\Delta$  urinary CPR (amount of decrease) was significantly greater during the non-HP + Ex period than

during the HP + Ex period. With regard to catecholamines, urinary adrenaline excretion levels were significantly higher during the HP + Ex period than during the non-HP + Ex period. Excretion levels of noradrenaline and dopamine were significantly increased during the HP + Ex period and significantly higher during the HP + Ex period compared with the non-HP + Ex period. The  $\Delta$  urinary noradrenaline and  $\Delta$  urinary dopamine (amount of increase) were significantly greater during the HP + Ex period than during the non-HP + Ex period.

### 3.4. CORRELATIONS AMONG BLOOD AND URINARY METABOLIC PARAMETERS

Spearman's correlation coefficients ( $\Delta$  during both the non-HP + Ex and HP + Ex periods [ $n = 14$ ]) were calculated to examine the relationships among glucose and lipid metabolism-related parameters (**Table 4**) and among protein-related parameters and urinary catecholamines (**Table 5**).



Table 4. Spearman's correlation coefficients among variables in terms of change ( $\Delta$ ) during both the non-HP + Ex and HP + Ex periods (n=14).  
(among the glucose and lipid metabolism-related parameters)

		①	②	③	④	⑤	⑥	⑦
① $\Delta$ HOMA-IR		—	0.122	0.331	0.355	0.400	– 0.149	<b>0.641*</b>
[ Serum ]								
② $\Delta$ TG	(mg/dl)		—	<b>0.918***</b>	– 0.463	– 0.482	– <b>0.672**</b>	0.180
③ $\Delta$ RLP-C	(mg/dl)			—	– 0.220	– 0.215	– <b>0.717**</b>	0.449
④ $\Delta$ TC	(mg/dl)				—	<b>0.924***</b>	0.475	<b>0.645*</b>
⑤ $\Delta$ LDL-C	(mg/dl)					—	0.329	<b>0.737**</b>
⑥ $\Delta$ HDL-C	(mg/dl)						—	– 0.207
⑦ $\Delta$ AI								—

\* p<0.05, \*\*p<0.01, \*\*\*p<0.001

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.

Table 5. Spearman's correlation coefficients among variables in terms of change ( $\Delta$ ) during both the non-HP + Ex and HP + Ex periods (n=14).  
(among the protein-related parameters and the urinary catecholamines)

		①	②	③	④	⑤	⑥	⑦	⑧	⑨	⑩
[ Serum ]											
① $\Delta$ UN	(mg/dl)	—	<b>744**</b>	0.514	0.526	<b>0.678**</b>	<b>0.810***</b>	<b>0.827***</b>	0.343	0.431	0.440
[ Plasma ]											
② $\Delta$ Valine	(nmol/ml)		—	<b>0.568*</b>	<b>0.543*</b>	<b>0.899***</b>	<b>0.785***</b>	<b>0.736**</b>	0.253	0.393	0.371
③ $\Delta$ Leucine	(nmol/ml)			—	<b>0.706**</b>	<b>0.783***</b>	<b>0.724**</b>	0.407	0.213	0.251	0.103
④ $\Delta$ Isoleucine	(nmol/ml)				—	<b>0.776**</b>	<b>0.666**</b>	0.415	– 0.077	0.086	– 0.112
⑤ $\Delta$ BCAA s	(nmol/ml)					—	<b>0.842***</b>	<b>0.618*</b>	0.196	0.354	0.152
⑥ $\Delta$ Lysine	(nmol/ml)						—	<b>0.723**</b>	0.112	0.442	0.165
[ Urine ]											
⑦ $\Delta$ UN	(mg/day)							—	0.336	<b>0.600*</b>	<b>0.692**</b>
⑧ $\Delta$ Adrenaline	( $\mu$ g/day)								—	<b>0.714**</b>	<b>0.596*</b>
⑨ $\Delta$ Noradrenaline	( $\mu$ g/day)									—	<b>0.653*</b>
⑩ $\Delta$ Dopamine	( $\mu$ g/day)										—

\* p<0.05, \*\*p<0.01, \*\*\*p<0.001

UN, urea nitrogen; BCAAs, branched chain amino acids.

The  $\Delta$  HOMA-IR and  $\Delta$  AI were significantly positively correlated ( $r = 0.641$ ,  $p < 0.05$ ). The  $\Delta$  serum TG was significantly positively highly correlated with  $\Delta$  serum RLP-C ( $r = 0.918$ ,  $p < 0.001$ ) and negatively correlated with  $\Delta$  serum HDL-C ( $r = -0.672$ ,  $p < 0.01$ ). Furthermore,  $\Delta$  serum RLP-C was significantly negatively correlated with  $\Delta$  serum HDL-C ( $r = -0.717$ ,  $p < 0.01$ ). In addition,  $\Delta$  serum TC was significantly positively highly correlated with  $\Delta$  serum LDL-C ( $r = 0.924$ ,  $p < 0.001$ ) and  $\Delta$  AI ( $r = 0.645$ ,  $p < 0.05$ ). The  $\Delta$  serum LDL-C was significantly positively correlated with  $\Delta$  AI ( $r = 0.737$ ,  $p < 0.01$ ).

The  $\Delta$  serum UN was significantly positively correlated with  $\Delta$  plasma valine ( $r = 0.744$ ,  $p < 0.01$ ),  $\Delta$  plasma BCAAs ( $r = 0.678$ ,  $p < 0.01$ ),  $\Delta$  plasma lysine ( $r = 0.810$ ,  $p < 0.001$ ) and  $\Delta$  urinary UN ( $r = 0.827$ ,  $p < 0.001$ ). Values for  $\Delta$  plasma valine, leucine, isoleucine, BCAAs and lysine were significantly positively correlated with each other ( $r = 0.543$  to  $0.899$ ,  $p < 0.05$  to  $0.001$ ). The  $\Delta$  urinary UN was significantly positively correlated with  $\Delta$  plasma valine ( $r = 0.736$ ,  $p < 0.01$ ),  $\Delta$  plasma BCAAs ( $r = 0.618$ ,  $p < 0.05$ ),  $\Delta$  plasma lysine ( $r = 0.723$ ,  $p < 0.01$ ),  $\Delta$  urinary noradrenaline ( $r = 0.600$ ,  $p < 0.05$ ) and  $\Delta$  urinary dopamine ( $r = 0.692$ ,  $p < 0.01$ ). The  $\Delta$  urinary adrenaline, noradrenaline and dopamine values were significantly positively correlated with each other ( $r = 0.596$  to  $0.714$ ,  $p < 0.05$  to  $0.01$ ).

## 4. Discussion

The present study examined the effects of exercise in combination with consumption of BCAA-rich fat-free milk or without consumption on protein-related, muscle synthesis- and breakdown-related, and glucose and lipid metabolism-related parameters, based on the hypothesis that serum insulin and urinary CPR excretion levels would increase during exercise when consuming fat-free milk compared with not consuming fat-free milk. We examined potential differences in muscle protein synthesis- and breakdown-related parameters and glucose and lipid metabolism-related parameters between subjects who consumed BCAA-rich fat-free milk and those who did not.

The primary results of the study were as follows. First, serum UN, plasma BCAAs and urinary UN excretion levels increased significantly with consumption of fat-free milk, whereas serum IGF-1 levels decreased significantly after both the non-HP + Ex and HP + Ex periods. Urinary CPR excretion levels were significantly lower during the non-HP + Ex period than during the HP + Ex period, but serum TG and RLP-C levels were significantly decreased after both the non-HP + Ex and HP + Ex periods. Furthermore, there were no significant differences in  $\Delta$  serum TG or  $\Delta$  serum RLP-C between the two periods. An unexpected result was that urinary dopamine and noradrenaline excretion levels were significantly greater during the HP + Ex period than during the non-HP + Ex period.

4.1. PROTEIN-RELATED AND MUSCLE PROTEIN SYNTHESIS AND BREAKDOWN-RELATED PARAMETERS  
Body weight, percent body fat, fat mass, and skeletal

muscle mass remained almost unchanged during both the non-HP + Ex and HP + Ex periods, suggesting that a period of 3 days was not sufficient for detecting changes in body composition.

The increases in serum and urinary UN and plasma valine and BCAA levels during the HP + Ex period are thought to reflect the effects of fat-free milk intake. However, why leucine and isoleucine levels did not significantly change is unclear, although they may have been utilized as energy sources or for body protein synthesis. Plasma lysine levels were significantly higher after the HP + Ex period than after the non-HP + Ex period, and  $\Delta$  plasma lysine (amount of increase) was significantly greater after the HP + Ex period than after the non-HP + Ex period. Lysine reportedly exerts a suppressing effect on muscle protein degradation.<sup>27</sup>

To examine muscle protein metabolism, we measured serum IGF-1, myostatin, and testosterone levels as markers of anabolism,<sup>28-30</sup> whereas serum and urinary cortisol levels were determined as markers of catabolism.<sup>30, 31</sup>

Serum IGF-1 levels were significantly decreased after both the non-HP + Ex and HP + Ex periods, and there was no difference in  $\Delta$  serum IGF-1 (amount of decrease). Nutritional factors such as energy and the quality and quantity of protein affect IGF-1 biosynthesis.<sup>32</sup> Regarding the effects of exercise, it is known that exercise activates IGF-1<sup>33</sup> and that the blood concentration of growth hormone, which promotes IGF-1 production, increases transiently during exercise.<sup>34</sup> In terms of acute effects, both endurance and resistance exercise reportedly increase total IGF-1 levels while leaving free IGF-1 unchanged.<sup>35</sup> In terms of the effects of training, total IGF-1 levels are reportedly higher in the elderly,<sup>36</sup> whereas they decrease up to the age of 40 and then increase after that age.<sup>37</sup> By contrast, other reports indicate that total IGF-1 levels decrease.<sup>38,39</sup> Schmits et al.<sup>38</sup> reported that 15 weeks of twice-weekly strength training in women aged 30-50 years reduces body fat percentage, fasting insulin, BG, and total IGF-1 levels, whereas levels of IGF-binding protein (IGFBP)-1 and -3 and free IGF-1 remain unchanged. Sterczala et al.<sup>39</sup> reported that 12 weeks of thrice-weekly strength training in women and men aged  $21.7 \pm 3.7$  years increases lean body mass and cross-sectional area of the thigh and decreases basal levels of serum IGF-1, free IGF-1, IGFBP-2 and 3, whereas levels of IGFBP-1 and 4 are increased. Regarding the mechanism underlying the exercise-induced decrease in serum IGF-1 levels, Arnarson et al.<sup>40</sup> hypothesized that "IGF-1 is redistributed from circulation into tissue during periods of active muscle building", whereas Sterczala et al.<sup>39</sup> hypothesized that "IGF-1's effect on muscle hypertrophy is mediated by a training-induced repartitioning of IGF-1 among its various binding proteins, both in circulation and in the interstitium". Although the mechanism is not fully understood, the findings of these studies suggest that in relatively young individuals, exercise training reduces serum IGF-1 levels, which in turn improves body composition and glucose metabolism. Therefore, the results of our previous<sup>20</sup> and current studies suggest that

the activity of IGF-1 is enhanced during exercise. However, it is unclear whether the effect of IGF-1 is amplified by the intake of fat-free milk.

No significant changes were observed in serum free-testosterone, myostatin, CK, cortisol, and urinary cortisol levels after the non-HP + Ex and HP + Ex periods. Testosterone is androgenic-anabolic hormone that promotes muscle growth. Although serum testosterone levels increases with acute exercise,<sup>41,42</sup> the effects of exercise are unlikely to be reflected in early morning, fasting samples, such as those examined in this experiment, and no clear exercise training effects are known.<sup>43</sup> Myostatin (also known as GDF-8 (growth differentiation factor-8)) is primarily synthesized in skeletal muscle, where it inhibits muscle growth. Therefore, a decrease in the myostatin expression level results in an increase in muscle mass.<sup>44</sup> It has also been shown that myostatin inhibition significantly alleviates the progression of obesity and diabetes, and exercise has been shown to reduce myostatin expression levels in skeletal muscle.<sup>45</sup> Plasma myostatin levels also reportedly decrease after 6 months of regular aerobic exercise,<sup>46</sup> but this effect may not be observed after only 3 days of exercise, as in this study. As serum CK levels did not increase significantly after either the non-HP + Ex or HP + Ex periods, it was thought that only a little muscle damage was incurred during the exercise in this study. Therefore, it was considered that cortisol was also not affected by the present experimental conditions.

3-MH, an indicator of the rate of muscle protein breakdown, is released into the blood and excreted in urine.<sup>47</sup> In our previous study,<sup>20</sup> urinary 3-MH excretion levels decreased during the fat-free milk intake period with no exercise and did not decrease during the fat-free milk intake period with exercise. Urinary 3-MH excretion levels were unchanged during both the non-HP + Ex and HP + Ex periods in the present study, suggesting that the muscle protein turnover rate was enhanced.<sup>48,49</sup>

#### 4.2. GLUCOSE AND LIPID METABOLISM-RELATED PARAMETERS

In our previous study,<sup>20</sup> urinary CPR excretion levels increased during the fat-free milk intake period with no exercise but showed no significant changes during the exercise period with fat-free milk intake. In the present study, urinary CPR excretion levels decreased significantly during the non-HP + Ex period but were unchanged during the HP + Ex period. An increase in insulin secretion due to protein intake<sup>13-15</sup> was also confirmed under the exercise conditions in this study. By contrast, there were no differences in serum insulin levels between the two periods. Decreases in the plasma and urinary excretion levels of CPR due to exercise have been reported elsewhere.<sup>50,51</sup> Serum TG and RLP-C levels were significantly decreased after both the non-HP + Ex and HP + Ex periods, and no significant differences were observed between the non-HP + Ex and HP + Ex periods in term of  $\Delta$  serum TG and  $\Delta$  serum RLP-C. Furthermore, a highly significant correlation was observed between  $\Delta$  serum TG and  $\Delta$  serum RLP-C. No differences were observed in the levels of the other serum lipids, HOMA-

IR, and AI between the non-HP + Ex and HP + Ex periods. Whether the intake of protein, particularly BCAAs, is associated with an increased risk of type 2 diabetes mellitus remains controversial.<sup>52,53</sup> The findings of our present study showed that insulin secretion is not spared but also not increased as a result of exercise with consumption of BCAA-rich fat-free milk. Furthermore, the effects on glucose and lipid metabolism appeared to be similar regardless of whether or not fat-free milk is consumed. However, excessively high protein intake is associated with various adverse effects.<sup>54</sup>

#### 4.3. URINARY CATECHOLAMINE EXCRETION

Urinary dopamine excretion levels were significantly increased only during the HP + Ex period and not during the non-HP + Ex period. Urinary excretion levels of dopamine, noradrenaline, and adrenaline were all significantly higher during the HP + Ex period compared with the non-HP + Ex period.

Dopamine deficiency is often discussed in terms of its psychological effects, as well as its connection to Parkinson's disease. From a physiological perspective, dopamine reportedly plays roles in the regulation of cardiovascular and renal function, as well as muscle tone, visual processing, calcium homeostasis, protein synthesis, and conceivably, the optimal utilization of food.<sup>55</sup> There are also reports that dopamine is involved in thermoregulation.<sup>56</sup> In our previous study,<sup>20</sup> female subjects consumed fat-free milk during both the non-exercise and exercise periods, and the energy expended through exercise was not replenished. As a result, urinary dopamine excretion did not change and did not differ between the two periods. In this study, the experimental conditions were set as with or without consumption of fat-free milk during the two exercise periods, and the energy expended through exercise was replenished. The significance and mechanism of the differences in urinary dopamine excretion and adrenal medulla/sympathetic nervous system function between the two exercise periods are unknown. But significant correlations were observed between  $\Delta$  urinary dopamine and  $\Delta$  urinary UN, suggesting that consuming fat-free milk during exercise under conditions of sufficient energy supply may increase dopamine production.

The present study, has several limitations. First, the study population was small, so a larger number of subjects is needed. Second, the subjects were middle-aged and older individuals for whom metabolic syndrome and sarcopenia are problematic, and it is thus necessary to determine whether there were any differences related to sex and age. Third, this was a short observational study that examined treatments for only 3 days. The effect of a longer experimental period needs to be examined, and the acute effects of exercise and long-term training must be examined in terms of indicators of body protein anabolism and catabolism. Fourth, the content of the diet and details of the exercise program should be considered. It is necessary to examine the dietary energy and protein supply conditions in addition to the intensity and duration of the aerobic and resistance exercise regimens.

## 5. Conclusion

A high-protein diet during exercise attenuates the insulin-sparing effects but does not affect the effects on glucose and lipid metabolism, although its effects on body protein synthesis are unclear. The combination of a high-protein diet and exercise is suggested to be an effective regimen for sarcopenic obesity.

**Conflicts of interest statement:** The authors have no conflicts of interest to declare.

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