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Increase in insulin secretion and decrease in muscle degradation by fat-free milk intake are attenuated by physical exercise

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ABSTRACT

Background: Protein intake, particularly branched chain amino acids (BCAAs), and exercise have opposing actions on insulin secretion, but the same action on protein anabolism. We examined the effects of BCAA-rich fat-free milk intake and/or exercise on levels of insulin secretion and indices related to muscle protein metabolism in order to assess the potency of dietary and exercise therapies against metabolic and locomotive disorders.
Methods: Eight adult female volunteers participated in all four 24 h experiments; control diet intake with or without exercise, and fat-free milk-containing diet intake with or without exercise. Fat-free milk was replaced with one-sixth of all foods in the control diet. Exercise was set at an equal-energy level as fat-free milk. Urine and fasting blood samples were collected for each experiment.
Results: Urinary C-peptide immunoreactivity excretion and serum insulin levels were significantly higher, but urinary 3-methyl-histidine excretion levels were significantly lower with low urinary adrenaline and dopamine excretion in the fat-free milk-containing diet than in the control diet. These findings were reduced by exercise with high urinary adrenaline and noradrenaline excretion.
Conclusions: BCAA-rich fat-free milk intake enhanced insulin secretion and suppressed muscle protein degradation, but these effects are attenuated by exercise accompanied with increase in catecholamine secretion.

1. Introduction

Metabolic syndrome has always been identified as a risk factor for coronary heart disease and stroke, which are closely linked to obesity and unhealthy lifestyle [1–3]. In contrast, the term “sarcopenia” was coined by Rosenberg in 1989 to denote the age-related decline in muscle mass and function [4,5], and recently, the concept of locomotive syndrome has been suggested in relation to bone disorder and sarcopenia [6,7]. Furthermore, sarcopenic obesity has been defined as a condition that encompasses sarcopenia and obesity [8,9]. Both nutrition and exercise are key factors involved in metabolic and locomotive syndrome [2,3,10,11]. Regarding metabolic syndrome, obesity, impaired glucose tolerance, hyperlipidemia and hypertension are frequently improved by energy-restricted diet and/or aerobic exercise, which directly promotes glucose and free fatty acid (FFA) consumption. During acute aerobic exercise, glucose transporter 4 increases glucose intake into muscle cells [12], serum FFA level increases [13], triglyceride (TG) level decreases [14] and lipoprotein lipase (LPL) activity is promoted [15]. The cumulative training effects of aerobic exercise are also understood [16,17]. Regarding locomotive syndrome, sufficient energy and adequate nutrients such as protein, n-3 polyunsaturated fatty acids, calcium and vitamin D, are required [9–11]. In addition, mechanically loaded and/or resistance exercise is typically used to increase bone mineral density and muscle mass [7,9–11], indirectly improving dysfunctional glucose and lipid metabolism.

It is known that insulin lowers blood glucose and stimulates protein anabolism. While it is necessary to avoid excessive secretion of insulin from the viewpoint of metabolic syndrome, insulin is also considered to contribute to increases in muscle mass. Exercise reduces insulin secretion [18] and promotes body protein anabolism [19,20]. On the other hand, proteins, particularly branched chain amino acids (BCAAs), are utilized for energy [21], body protein maintenance [22] and anabolism [23], but simultaneously produce insulinotropic effects [24–26]. Thus, BCAA intake and exercise have opposing actions on insulin secretion, but the same action on protein metabolism. Therefore, it is significant to investigate each and combined effect of BCAA intake and exercise.

In the present study, we examined the effects of BCAAs-rich fat-free milk intake or exercise, and the effects of exercise under fat-free milk intake on serum insulin, urinary C-peptide immunoreactivity (CPR) excretion and indices related to muscle protein metabolism in order to

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assess the potency of dietary and exercise therapies against metabolic and locomotive disorders.

2. Materials and methods

2.1. Subjects

Eight healthy adult female volunteers [age, 22 ± 0 years; height, 159 ± 1 cm; weight, 52.8 ± 2.4 kg; mean ± standard error of the mean (SEM)] provided written, informed consent to participate in all procedures associated with the study, which proceeded according to the Declaration of Helsinki (1964; amended 2013).

2.2. Experimental procedure

The Research Ethics Committee of Kanto Gakuin University approved the study.

2.2.1. Experimental protocol

The study comprised four experiments. Eight subjects participated in all four experiments in random order; control diet intake, control diet intake with exercise, fat-free milk-containing diet intake and fat-free milk-containing diet intake with exercise. Each experiment was conducted at interval of one week or more. Participants were fed experimental diets in all four experiments, and exercised in two experiments under the control diet or fat-free milk-containing diet. In every experiment, the participants ate the prescribed supper on the previous evening and stayed two nights at the metabolic unit to complete each experiment.

2.2.2. Experimental diet

For control diet intake, the diet comprised the following foods; soft rolls, sausages (Vienna), ketchup, potato salad, vegetable juice, spaghetti, salted cod sauce, sweet corn (boiled), salted butter, pepper (mixed, ground), vitamin mixture beverage, custard pudding, European plums (dried), well-milled rice, soy protein processed food, miso (light-yellow type), kanze-fu, spinach (leaves, boiled) and soy sauce. The composition of the experimental diet was based on the recommended dietary allowance or adequate intake published in the Dietary Reference Intakes for Japanese [27]. The calculated values per day on the Standard Tables of Food Composition in Japan [28] were as follows: energy, 2100 kcal; protein, 63.8 g (BCAAs, 9.9 g); lipid, 67.8 g; carbohydrate, 302.2 g and the PFC ratio was 12.2:29.1:58.7. For fat-free milk-containing diet intake, the experimental diet was reduced to approximately five-sixths of control diet by reducing all foods in proportion to the energy intake, and fat-free milk was replaced with approximately one-sixth of control diet. Fat-free milk was given with every three meals and its volume was decided as the likely level in daily life, i.e., equivalent energy amount in 175 ml of ordinary milk per meal. The participants consumed experimental diets (energy, 2100 kcal; protein, 88.9 g (BCAAs, 15.3 g); lipid, 57.0 g; carbohydrate, 302.3 g and the PFC ratio was 17.4:24.5:58.2) that contained fat-free milk at 352 kcal.

In control diet intake with exercise, no extra energy corresponding to addition energy expended during exercise was added. In fat-free milk-containing diet with exercise, the participants consumed control diet and 352 kcal of fat-free milk corresponding to extra energy (energy, 2452 kcal; protein, 99.6 g (BCAAs, 17.0 g); lipid, 68.4 g; carbohydrate, 352.9 g and the PFC ratio was 16.6:25.1:58.2).

The calculated values of other amino acids (lysine, sulfur amino acids (methionine, cystine: SAAs), aromatic amino acids (phenylalanine, tyrosine: AAAs)) relatively relevant to protein intake in each experiment were as follows: lysine, 2.8 g; SAAs, 2.2 g; AAAs, 4.8 g in control diet intake and control diet intake with exercise, lysine, 4.9 g; SAAs, 2.9 g; AAAs, 7.2 g in fat-free milk-containing diet intake, lysine, 5.3 g; SAAs, 3.3 g; AAAs, 8.0 g in fat-free milk-containing diet intake with exercise.

2.2.3. Procedure of exercise

Exercise tolerance was tested before starting the study by gradually increasing the work rate on a bicycle ergometer. Heart rate was recorded by telemetry (DS-3400; Fukuda Denshi Co., Ltd., Tokyo, Japan). Expired gas before and at steady state during exercise was gathered using a Douglas bag, and then gas volume was measured using a dry gas meter (DC-5A; Shinagawa Corporation, 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). Relationships among additional energy expenditure calculated based on oxygen intake and the respiratory exchange ratio, work rate (kilopond of bicycle ergometer) and heart rate were determined for each individual.

In the exercise experiment, participants expended 352 kcal by pedaling a bicycle ergometer at a target intensity of 40–50% of maximal oxygen intake for 101 ± 4 min split between the morning and afternoon. In the non-exercise experiment, the participants performed only normal daily activities.

2.2.4. Environmental conditions

Room temperature during the experimental period was maintained between 25 and 26 °C on the dry-bulb thermometer and between 22 and 23 °C on the wet-bulb thermometer.

2.2.5. Sample collection and measurement

Nitrogen contents of breakfast, lunch, supper and fat-free milk were determined by the Kjeldarl method. Urine samples were collected at 6:00–8:00 (baseline before each experiment), 8:00–14:00, 14:00–20:00 and 20:00–8:00 the following morning to measure creatinine [29], urea nitrogen (UN) [30], CPR (Chemiluminescent enzyme immunoassay, Lumipulse Presto C-peptide; Fujirebio Inc., Tokyo, Japan), 3-methylhistidine (3-MH) [31], adrenaline, noradrenaline and dopamine [32] levels. Fasting blood samples were collected early in the morning at the end of each experiment to measure blood glucose (BG) [33], serum immunoreactive insulin (IRI) [34], FFA [35], TG [36], remnant-like particle-cholesterol (RLP-C) [37], UN [30], insulin-like growth factor-1 (IGF-1: Immunoradiometric assay, IGF-1 (Somatomedin C) IRMA DAIICHI; Fujirebio Inc., Tokyo, Japan) and cortisol (Electro chemiluminedcence immunoassay, ECLusys reagent Cortisol II; Roche Diagnostics K. K., Tokyo, Japan) levels. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated by multiplying fasting BG (mg/dl) by IRI (μU/ml) by 1/405.

2.3. Statistical analysis

Results are expressed as means ± SEM. All statistical analyses were performed using SPSS14.0 for Windows. We applied the Wilcoxon signed-ranks test to examine differences between control diet intake and control diet intake with exercise, fat-free milk-containing diet intake, fat-free milk-containing diet intake with exercise, and between fat-free milk-containing diet intake with or without exercise. Significance was established at $p < 0.05$ in all analyses.

3. Results

There was no significant difference in the timing of the menstrual cycle in each experiment (when the whole menstrual cycle was taken as 100%, at 45 ± 10 percentile point, control diet intake; at 67 ± 11 percentile point, control diet intake with exercise; at 69 ± 10 percentile point, fat-free milk-containing diet intake; at 57 ± 9 percentile point, fat-free milk-containing diet intake with exercise).