Effect of Light Resistance Exercise after Ingestion of a High-Protein Snack on Plasma Branched-Chain Amino Acid Concentrations in Young Adult Females

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(Received July 16, 2008)

Summary  We have previously reported the preventive effects of light resistance exercise (voluntary tower climbing exercise) after ingestion of a high-protein snack (HPS) on muscle loss in glucocorticoid-injected rats. However, such studies have not been performed in humans. In this cross-over study, we examined the effect of light resistance exercise after ingestion of HPS on plasma branched-chain amino acid (BCAA) concentrations in humans. Seven healthy young adult females (aged 22.1±1.2 y) participated in this study. They were assigned to either an exercise group or a control group. Seven days after the first experiment, they were crossed over to the opposite intervention. The subjects ingested HPS (15 g protein, 18 g sugar) 3 h after breakfast (basal meal). The plasma BCAA concentrations increased at 30 min after HPS ingestion. The subjects in the exercise group performed light resistance exercise (15 min dumbbell exercise using 300 g brown-rice-filled fabric dumbbells) when the plasma BCAA concentrations were increased (60 min after the snack ingestion). The control group maintained a resting position during the experiment. Changes in the plasma BCAA concentrations between 60 and 90 min after HPS ingestion increased continuously in the control group (+27 µmol/L) but decreased in the exercise group (−37 µmol/L). Therefore, light resistance exercise after HPS ingestion may be effective for utilization of plasma BCAA in humans.

Key Words  high-protein snack, light resistance exercise, branched-chain amino acids, muscular blood flow, dumbbell exercise

Moderate exercise and protein intake are essential for maintaining and increasing skeletal muscle mass. Many studies have been conducted on the timing of exercise and protein or amino acid intake for efficiently increasing skeletal muscle mass (1–5). In particular, branched-chain amino acid (BCAA) accounts for about 35% of the essential amino acids that constitute muscle protein (6), and leucine has been investigated for its protein anabolic and insulin-like effects (7, 8).

We have previously reported that the serum amino acid concentrations are not significantly increased after ingestion of basal meals, whereas the concentrations are rapidly increased after high-protein snack (HPS) ingestion in rats (9). It was considered that ingestion of HPS approximately 3 h after the basal meals is an effective method to supply muscles with sufficient amounts of amino acids (10).

To examine whether the ingestion of HPS is effective in supplying amino acids to muscle and suppressing muscle loss, we have previously studied this phenomenon in glucocorticoid-injected rats (9, 11). We found that muscle loss cannot be suppressed by providing HPS alone without light resistance exercise (9, 11).

In this study, we examined the effect of light resistance exercise after HPS ingestion 3 h after a basal meal on plasma BCAA concentrations and estimated whether the ingestion of HPS and light resistance exercise was effective for utilization of BCAA in humans.

SUBJECTS AND METHODS

1. Subjects. Seven healthy young adult females participated in this study. They were randomly assigned to either an exercise group or a control group. Seven days after the first experiment, they were crossed over to the opposite intervention. The subjects gave their consent regarding the purpose of the study, study methodology, and publication of the study results. This study was implemented after permission from the Waseda University Ethics Committee.

2. Experimental protocol. The subjects were prohibited from eating or drinking excessively and doing exercise on the day before the experiment. Eating or drinking, except for water, was prohibited for 12 h prior to the start of the experiment.

On the experimental day, the subjects were provided with breakfast (basal meal) and HPS 3 h after the basal meal. The subjects in the exercise group performed dumbbell exercise for 15 min, 60 min after HPS inges-
tion. The control group maintained a resting position during the experiment (Fig. 1). Blood was collected before the basal meal (at -180 min), before HPS ingestion (at 0 min) and at 30, 60, 90, and 120 min after HPS ingestion (Fig. 1).

3. Breakfast (basal meal). Composition of the breakfast was toast, milk, cornflakes, cheese, ham, orange juice, and fruit jelly. The energy and protein content of the breakfast were calculated by equally dividing daily total energy (35 kcal·kg⁻¹·d⁻¹) and protein (1.0 g·kg⁻¹·d⁻¹) into three, after subtracting the portion of the HPS.

4. High-protein snack. High-protein snack was made of dried egg whites (51 kcal/14.5 g) (Q.P. Corporation, Tokyo, Japan), gelatin (2.5 g), sugar (18.0 g) and water and consisted of 15 g protein and an energy content of 130 kcal.

The amount of the protein of HPS was adjusted to 15 g, which is 30% of the recommended dietary allowance (RDA) for Japanese females.

5. Exercise. The subjects performed dumbbell exercises invented by Suzuki as a light resistance exercise (12, 13), using a pair of dumbbells made of fabric packed with 300 g of brown rice, 60 min after the snack ingestion. Dumbbell exercises were performed as follows: the subjects stood on the floor with the feet shoulder-width apart, stretched the back, bent the upper body forward, bent the knees, and maintained the half-crouching position as a basic position. Subse-

Table 1. Characteristics of the subjects.

| Characteristic     | Value     |
|--------------------|-----------|
| Age (y)            | 22.1 ± 1.2|
| Height (cm)        | 156.4 ± 4.3|
| Weight (kg)        | 55.4 ± 7.0|
| Body fat (%)       | 28.7 ± 3.4|
| BMI (kg/m²)        | 22.6 ± 2.0|

Values are means ± SD (n=7).

Table 2. Program of dumbbell exercise.

| Exercise | Description                          |
|----------|--------------------------------------|
| 1        | Standing shoulder press              |
| 2        | Bent dumbbell row                    |
| 3        | Squat                                |
| 4        | Upper body twist                     |
| 5        | Butterfly                            |
| 6        | Bent lateral raise                   |
| 7        | Simultaneous curl                    |
| 8a       | Concentration curl (right)           |
| 8b       | Concentration curl (left)            |
| 9a       | One hand draw up (right)             |
| 9b       | One hand draw up (left)              |
| 10a      | Kickback (right)                     |
| 10b      | Kickback (left)                      |
| 11       | Front dumbbell raise                 |
| 12       | Arm extension                        |

Fig. 1. Experimental protocol.

Fig. 2. Responses of plasma BCAA (A), glucose (B), and insulin (C) concentrations to dumbbell exercise after the ingestion of basic meals and HPS. Values are means ± SD (n=7). *Significantly different from time 0 (Exercise group) (p<0.05). †Significantly different from time 0 (Control group) (p<0.05).
sequently, they grasped the dumbbells firmly; twisted the wrists inward and kept them curled. The exercise program comprised 12 exercises (Table 2). Each movement was repeated 15 times. The interval of each exercise was about 7 s. Subjects moved the dumbbells slowly (2–3 s for eccentric and concentric actions) and continuously.

6. Analysis of blood components. Blood samples were collected and plasma was analyzed for BCAA and insulin (Mitsubishi Chemical Medience, Co. Ltd., Tokyo, Japan). Plasma glucose was analyzed using the glucose CII test (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

7. Muscle blood flow. Changes in total hemoglobin content in the forearm and the vastus lateralis muscles were measured during the dumbbell exercises using near infrared spectroscopy (Hamamatsu Photonics, Co. Ltd., Shizuoka, Japan) to confirm the fluctuation in skeletal muscle blood flow (14).

8. Statistical analysis. All data were expressed as means±SD. Concentrations of plasma components were analyzed by Bonferroni’s multiple comparison test after two-way ANOVA. t-tests were used to measure significant differences in changes in plasma components 60–90 min after HPS ingestion, and in hemoglobin contents before and after dumbbell exercise. Levels of significance of less than 5% were considered statistically significant.

RESULTS

1. Plasma BCAA concentrations

Plasma BCAA concentrations were significantly higher at 30, 60, 90, and 120 min after HPS ingestion, compared with the point immediately before HPS ingestion (at 0 min), in both the exercise and control groups. In the control group, plasma BCAA concentration continually increased for 90 min after HPS ingestion. In the exercise group, plasma BCAA concentrations decreased during the dumbbell exercise (Fig. 2A). There was a significant difference in plasma BCAA concentrations in the exercise group compared with the control group between 60 and 90 min, with plasma BCAA concentrations declining significantly after exercise (Fig. 3A).

2. Plasma glucose concentrations

Plasma glucose concentrations increased after HPS ingestion with a peak at 30 min. A subsequent rapid decrease of the concentrations until 60 min was followed by an increase again in both groups (Fig. 2B). The increase in plasma glucose concentrations was smaller in the exercise group compared with the control group but was not significantly different (Fig. 3B).

3. Plasma insulin concentrations

Plasma insulin concentrations increased after HPS ingestion with a peak at 30 min (Fig. 2C). Subsequently, the concentrations rapidly decreased until 60 min after HPS ingestion. The change patterns were similar and there was no significant difference between the two groups (Fig. 3C).

4. Muscle blood flow

Change in blood flows in the forearm and the vastus lateralis muscles after dumbbell exercise was significantly increased compared to that before the exercises (Fig. 4). Fluctuations in blood flows in the forearm and vastus lateralis muscles during dumbbell exercises were confirmed (Fig. 5).

DISCUSSION

We previously reported that the serum amino acid
concentrations were not significantly increased after ingestion of basal meals, whereas the concentrations rapidly increased after HPS ingestion in rats (9). It is conceivable that HPS should increase the amino acid supply to the peripheral tissues (10). This study showed that the concentrations of plasma BCAA significantly increased after HPS ingestion in humans. We also confirmed that the plasma BCAA concentrations were not significantly increased after ingestion of basal meals in a pilot study (data not shown). These results are consistent with our previous studies in rats (9).

This study confirmed that the plasma BCAA concentrations were significantly increased at 30 min after HPS ingestion and continued to increase in the control group, whereas the plasma BCAA concentration was decreased between 60 and 90 min in the exercise group (+27 μmol/L vs. −37 μmol/L). This suggests that light resistance exercise after HPS ingestion may be effective for amino acid utilization in humans.

High plasma amino acid concentrations increase the transport of amino acids to skeletal muscle cells and stimulate skeletal muscle protein synthesis in humans (15, 16). When human subjects receive an amino acid infusion into the antebibrachial vein, the net synthesis of muscle protein is increased due to increased amino acid transport into muscle cells (16). Similarly, when human subjects received oral amino acids, muscle protein synthesis is stimulated (17). Thus an increased plasma amino acid concentration, irrespective of the administration route, increases the transport of amino acids to muscle cells and stimulates synthesis of muscle protein. In this study the plasma BCAA concentration significantly increased after ingestion of HPS 3 h after ingestion of basal meal. We confirmed that the ingestion of HPS increases the plasma BCAA concentration, leading to an increase in amino acid supply to the muscles.

There have been some reports of subjects who have ingested either essential amino acids alone or a mixture of essential and non-essential amino acids after resistance exercise (18, 19). In both groups, changes in the balance between net synthesis and catabolism of muscle protein were almost equal, suggesting that the essential amino acids influence the promotion of muscle protein synthesis (18, 19). Volpi et al. administered either essential amino acids alone or a mixture of essential and non-essential amino acids orally to compare the metabolic responses of muscle protein synthesis in humans (20). As a result, they showed that the stimulatory effects of the administered amino acids on net synthesis of muscle protein were equal under both conditions (20). In this study, HPS was prepared from dried egg white (21) with sugar to promote insulin secretion (22, 23). Egg white is regarded as a high quality, nutritious food because it contains well-balanced levels of essential amino acids and is thus suitable as the protein component of a snack to promote muscle protein synthesis.

There are several studies on the effects of timing the administration of protein or amino acid on muscle protein metabolism (1–5). In one study, protein supplements were administered to human subjects immediately or 2 h after resistance exercise for 12 wk (three times per week) (1). The effects on muscle hypertrophy and muscle strength were greater when the protein supplements were administered immediately after exercise (1). The effects of oral amino acids administered immediately before or after resistance exercise on muscle protein anabolism were also studied (2). When the amino acids were administered immediately before exercise, the total uptake of blood amino acids and net muscle protein synthesis by leg muscles were significantly greater than after exercise (2). These results indicate that ingestion of amino acids immediately before exercise promotes amino acid uptake through an increase in muscle blood flow during exercise and promotes muscle protein synthesis.

Biolo et al. reported that an increase in muscle blood flow after exercise increases the transport of free amino acids to muscle cells and promotes muscle protein synthesis (15). In the exercise group of our study, dumbbell exercise, as light resistance exercise, was performed for 15 min, 60 min after HPS ingestion, at the peak period of plasma BCAA concentrations. The plasma BCAA concentration was significantly lowered by dumbbell exercise compared with the control group. Although dumbbell exercise is of light intensity, similar to walking...
(12), the blood flow in the forearm and vastus lateralis muscles have been confirmed to increase significantly 30 s after exercise compared with before exercise, and fluctuation in the blood flow during exercise has also been confirmed. It is conceivable that the increase and fluctuation in muscle blood flow may have stimulated the amino acid utilization (24).

Matsumoto et al. reported increased blood BCAA concentrations from BCAA ingestion during cycling exercise at 50% of maximum intensity with enhanced BCAA uptake in muscles (25). Another report revealed that oral BCAA administration before exercise increased ammonia production in muscles, reduced the amount of essential amino acids released from the muscles and suppressed endogenous muscle protein catabolism (26).

Based on the reports above, this study suggests the following two possibilities. The plasma BCAA concentration, which was increased by HPS ingestion, was lowered by dumbbell exercises through uptake by muscles for either utilization in muscle protein synthesis or for oxidation as an energy source, and inhibited endogenous muscle protein catabolism.

This study indicates that light resistance exercise after HPS may be effective in promoting plasma BCAA utilization in humans. However, it remains unclear whether the reduced plasma BCAA was used for muscle protein synthesis or for oxidative catabolism for energy generation. This needs to be clarified by further studies. It is also important to determine if a long period of HPS ingestion and light resistance exercise is effective in increasing muscle strength or muscle mass, based on the results of this study. Finally, the subjects in this study were young adult females, but further studies including elderly people are necessary.

Acknowledgments

This work was supported by Q.P. Corporation, Tokyo, Japan.

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