Protein Supplementation of a High-Carbohydrate Diet during Resistance Training Enhances Muscle Mass and Function by Increasing Essential Amino Acids

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Abstract

Objective: Previous studies have presented the effects of carbohydrate and protein intake using a single food or supplement. However, little knowledge is available regarding the long-term effects of protein diet supplementation on muscle mass and function. This study was conducted to investigate the effects of a 12-week high-intensity resistance exercise program combined with a high-carbohydrate (HCHO), high-protein (HPRO) diet on body composition, muscle function, anabolic/catabolic hormones, and blood amino acid levels.

Methods: This study included 27 male college students, who were divided into an HPRO group (n=12) and an HCHO group (n=15). Three to five sets of resistance exercises were performed four times a week at 75% of 1-repetition maximum for 12 weeks.

Results: The weight and body fat percentage decreased in both groups after the 12-week resistance exercise, and muscle mass increased in the HPRO group. The peak torque increased in the HPRO group, and the average power increased in both the HCHO and HPRO groups, although there was no significant difference between the changes in both groups. However, the testosterone level and the ratio of testosterone to cortisol increased in the HCHO group, and the changes were significantly different between the groups. The blood essential amino acid (EAA) and non-essential amino acid levels showed a time × group effect. Conclusion: Protein supplementation of a high-carbohydrate diet during resistance training may enhance body composition and muscle mass and function by increasing the blood EAA levels. Therefore, considering that Asians tend to consume high-carbohydrate diets, a diet with slightly less carbohydrate and increased protein may be more effective for increased muscle strength and mass during training.

Keywords: Resistance training; Body composition; Muscle function; Anabolic/catabolic hormone; Blood amino acid levels; Carbohydrate diet; Protein diet

Introduction

Resistance exercise promotes anabolic activity for muscle protein synthesis (MPS). Supplemental protein intake facilitates MPS during resistance training and induces a positive net protein balance [1], this is a positive algebraic event when protein synthesis is greater than protein breakdown [2,3]. During resistance exercise, athletes are generally required to consume more protein for restoration and reformation of damaged muscle [4].

The increased intake of protein plays an important role in improving muscle size and strength. It has been reported that a high protein intake (1.6-1.8 g/kg/day) improves body composition, enhances lean body mass [5], and affects anabolic hormones that are related to muscle remodelling [6]. Previous studies have reported that resistance exercise stimulates the secretion of anabolic growth hormone and testosterone [4], which stimulates MPS [7]. During resistance exercise, a high protein intake reportedly increases resting testosterone, cortisol, growth hormone, and insulin levels [4]. In contrast, other studies suggested that these hormones were unrelated to the magnitude of the MPS response or total muscle hypertrophy with resistance training [8,9]. Therefore, it is unclear whether changes in these hormones play a role in anabolic activity.

It has also been suggested that consuming proper amounts of protein and carbohydrates to meet the need for increased energy requirements during exercise training can prevent protein degradation, as well as reduce body fat and increase muscle mass and strength [10]. Replenishing carbohydrates during exercise maintains or increases the body's carbohydrate oxidation rate [11], maintains the blood glucose level [12], helps reduce the rate of perceived exertion [13], and slows glycogenolysis in the liver [14] and muscles [15], thus delaying the onset of fatigue during exercise [11]. It has also been reported that consuming additional carbohydrates after exercise increases muscle glycogen synthesis and is thus positively correlated with glycogen retention per hour during recovery [16].

Previous studies have reported that supplementing carbohydrate with additional protein facilitates the storage of muscle glycogen after exercise through the synergistic effect of carbohydrates and protein on insulin secretion [17,18], significantly increases lean body mass [19], and improves blood lipids [20]. Moreover, Cribb et al. [21] reported...
that the simultaneous intake of creatine, protein, and carbohydrates more significantly increased the muscle mass and strength than the intake of protein alone. Thus, the simultaneous intake of carbohydrates and protein was more effective for protein synthesis and inducing a positive net protein balance than a high-protein or high-carbohydrate diet alone during resistance exercise.

However, glucose alone had no significant effect on MPS, although glucose added to protein stimulates the net MPS [22]. Nevertheless, Jenjens et al. [23] suggested that the effect of additional protein intake on muscle mass was due to the greater number of calories provided by the carbohydrates and protein compared with that of carbohydrate intake alone; further, if adequate carbohydrates were provided, the addition of protein would have no beneficial effect on muscle hypertrophy.

Asians have high carbohydrate intake rates of more than 50-60%, with rice being their staple food. Thus, they only need to increase their protein intake during resistance exercise. Previous studies have shown the effects of carbohydrate and protein intake using a single food or supplement [24-26]. Little knowledge is available regarding the long-term effects of protein supplementation on muscle mass and function. In addition, the interactive effects of resistance training and high-protein diets on hormonal responses and protein balance are less clear and remain controversial [27-30].

Therefore, this study investigated whether protein supplementation of a typical Asian diet would increase muscle mass and function as represented by blood amino acid levels during resistance training, compared with the effects of an isocaloric high-carbohydrate diet.

Methods

Subjects

Twenty-seven healthy collegiate men were recruited via flyers posted around the Dankook University Campus and the Chungnam area. The subjects were included in accordance with the following specific criteria: a) no intake of muscle growth agent, supplement, or steroid that could affect muscle strength for 12 weeks before the start of this investigation and during the study, and b) healthy male college students who had not been diagnosed with musculoskeletal system disorder or metabolic diseases. The present study was approved by the Institutional Ethics Committee of Physical Education of Dankook University.

Experimental protocol

This study classified subjects by adjusting the protein intake ratio in the recommended dietary allowances for Koreans. The subjects were randomly assigned to the high-carbohydrate group (HCHO; age, 24.38 ± 1.68 years), in which they were asked to consume carbohydrates as more than 60% of their total energy intake (total calories: 1,924 kcal/day; 65.5% carbohydrate, 20.2% protein, and 14.3% fat) or high-protein group (HPRO; age, 24.43 ± 1.51 years), in which they were asked to consume carbohydrates as less than 50% of their total energy intake and more than 2.0 g/kg/day of protein (total calories: 1,904 kcal/day, 48% carbohydrate, 35.2% protein, and 16.8% fat). The study dietician advised each subject as to how they could achieve the goal intake of protein and other macronutrients, after analyzing their 3-day dietary recall prior to the initiation of the study. At the beginning of this study, 60 male college students agreed to follow the revised diet and wished to participate in the program; they were then divided into two groups. They were asked to maintain their usual physical activity and dietary intake, which were monitored, and exogenous variables other than resistance exercise were controlled. However, many subjects were excluded owing to personal reasons, changes in the extent of their physical activity, and irregular dietary intake. Twelve subjects in the HPRO group and 15 in the HCHO group who maintained their usual physical activity and dietary intake were finally analyzed. The subjects were all male college students because of the need to consider differences in muscle mass and strength based on sex.

Anthropometric measurement

Height and weight in light clothing were measured before and after the 12-week exercise training program. Body mass index (BMI) was calculated by dividing weight (kg) by the square of height (m). Body fat percentage and fat-free mass were measured using the BOD POD (MedGadget, Life Measurement, Inc., Germany); body volume and density were determined using air displacement and residual lung volume.

Resistance exercise program

The resistance exercise program involved the simultaneous use of free weights and machines to exercise the chest, back, shoulders, triceps, biceps, abdomen, and lower extremity muscle groups and was performed four times a week for 12 weeks. The resistance exercise program was formulated on the basis of previous studies [31], in which two to five sets of low- (8-10 repetition maximums (RMs)) to high-intensity (2-6 RM) resistance exercise increased maximal strength, after consultation with two in-service athletic trainers. This study used a resistance exercise method that began with 8-10 RMs and a maximal strength test for each exercise region (1RM), with increased load in the final set, and an exercise load method that increased the RM and sets at 4-week intervals. The abdominal exercise involved all-out exertion for 1 minute. The resting time between sets was 1-2 minutes.

Isokinetic strength test

The peak torque and average power of the thigh were measured using isokinetic strength equipment (Biodex 2000; D&A, Germany). The test was conducted on the basis of previous studies [32]. The subjects were asked to master the procedure and purpose of the measurement to enable them to exert maximal effort and to repeat five bouts of warm-ups before measurement. The maximal strength and muscular power were measured five times at 60°/s, and the subjects were encouraged to exert maximum effort.

Energy intake analysis

Dietary intake was surveyed using the estimated food record method for a total of 3 days (2 weekdays and 1 weekend day) before and after the 12-week resistance exercise program. Auxiliary material, cuisine, recipes, and serving and total food quantities were recorded. Weekly dietary and energy intake was analyzed using the 3.0-version Computer-aided Nutrient Analysis Program developed by the Nutritional Information Center of the Korean Nutrition Society. Subjects were asked to maintain a daily food intake of 1,800-2,000 kcal based on the recommended daily food intake for male Koreans in their 20s. Dietary intakes were individually prescribed by designing menus with reduced animal fat and modified carbohydrate and protein levels.
The recommended dietary protein sources were beans, boiled eggs, chicken breast, and other meat. Considering that carbohydrates account for 50-60% of the diet of most Koreans, the menu was adjusted to reduce only the fat intake and to maintain the protein intake in the HCHO group, and to reduce the carbohydrate intake by ≥10% and increase the protein intake in the HPRO group (Table 1).

| Variables          | HCHO        | HPRO        | Time | Time × Group |
|--------------------|-------------|-------------|------|--------------|
| Kcal/day           | 1773.6 ± 140.3 | 1924.9 ± 116.2 | 1911.2 ± 210.4 | 1903.6 ± 181.5 | 0.001 | 0.618 |
| CHO (g)            | 253.0 ± 20.3  | 291.9 ± 23.3**#  | 241.9 ± 16.7  | 218.9 ± 16.4*  | 0.003 | 0.006 |
| CHO (%)            | 59.5 ± 2.3    | 65.5 ± 1.8**#   | 57.8 ± 4.3    | 48.0 ± 3.2**   | 0.037 | 0       |
| Protein (g)        | 94.4 ± 11.3   | 99.6 ± 8.5**#   | 103.7 ± 13.1  | 160.3 ± 13.7***| 0      | 0.869   |
| Protein (g/kg)     | 1.3 ± 0.2     | 1.4 ± 0.1#      | 1.3 ± 0.2     | 2.0 ± 0.1**    | 0      | 0.01    |
| Protein (%)        | 22.4 ± 1.9    | 20.1 ± 1.6**#   | 24.7 ± 1.7    | 35.2 ± 3.1**   | 0      | 0.018   |
| Fat (g)            | 63.8 ± 10.5   | 63.6 ± 5.3#     | 74.6 ± 22.9   | 76.9 ± 17.8    | 0.158  | 0.179   |
| Fat (%)            | 15.2 ± 2.3    | 14.3 ± 1.2**#   | 17.5 ± 4.2    | 16.8 ± 3.2     | 0      | 0.044   |

Values are presented as means ± standard deviations. *Difference between the values at baseline and during the 12-week dietary recommendation p<0.01, **p<0.001
*Difference between the HCHO and HPRO groups after the 12-week resistance training. HCHO: High-Carbohydrate intake group; HPRO: High-Protein intake group; CHO: Carbohydrates.

Table 1: Macronutrient intake at baseline and during the 12-week resistance training.

Blood analysis

A blood test was obtained at 9:00 AM after a 12-hour fast. Subjects were asked avoid any physical activity of more than medium intensity for 24 hours before the test. Blood was collected from brachial veins using a disposable syringe. Plasma was separated using a centrifuge and was stored in a freezer at -70°C until it was analyzed. Growth hormone levels were measured using an immune-radiometric assay (Modular Analytics E170, Roche, Germany); Serum insulin (Modular Analytics) and testosterone levels (r-counter, COBRA 5010 Quantum, USA) were analyzed using commercially available human enzyme immunoassay kits. The cortisol level was measured with a radioimmunoassay kit using the two-antibody competitive reaction method (Modular Analytics). The blood amino acid level was measured using high-performance liquid chromatography, and the output material was detected, recorded over time, and quantified by peak area or height. The blood creatinine level was measured using the Jaffe reaction (Advia 1650, Bayer, Japan). The blood nitrogen level was measured using the glutamate dehydrogenase (GLDH) test method.

Urine analysis

To measure nitrogen and creatinine excretion, 24-hour urine was collected before and after the 12-week exercise. The level of nitrogen in the urine was analyzed with the GLDH test method using a blood urea nitrogen reagent kit (Advia). The urine creatinine level was analyzed by the Jaffe reaction method using a creatinine reagent kit (Advia).

Statistical analysis

To investigate differences between the groups before and after the 12-week resistance exercise program, two-way repeated-measures analysis of variance (intervention × time) was conducted; to investigate the percentage of changes in the variables, in which the difference between values at baseline and 12 weeks was divided by the baseline value and multiplied by 100, a paired t-test was conducted. Between-group comparisons were performed using an independent t-test and within-group comparisons used a paired t-test for post-hoc analysis. All statistical analyses were performed using SPSS-PC for Windows (version 14.0; SPSS Inc., Chicago, IL, USA), and p-values <0.05 were considered statistically significant.

Results

Average daily dietary intake

Calorie intake showed a significant time effect (p<0.01) after adjustment of the menu. The carbohydrate intake (g and percent of total intake) showed a significant time effect (p<0.05) and time × group effect (p<0.01). The protein intake (g, g per body weight, and percent of total intake) and fat intake (g and percent of total intake) also showed a significant time effect (p<0.001) and time × group effect (p<0.05) (Table 1).

Body composition changes

There were no significant differences in body composition between the groups before training. Body weight (p<0.001), BMI (p<0.05), and body fat percentage (p<0.01) showed a significant effect. Based on the post-hoc analysis, both the HCHO and HPRO groups showed a significant reduction in weight (p<0.05 vs. p<0.01) and body fat percentage (p<0.05 vs. p<0.05, respectively); further, the HPRO group showed a significant increase in muscle mass (p<0.05). However, there was no significant difference between the amounts of change in body composition after the 12-week resistance exercise program (Figure 1).

Changes in muscle function

There were no significant differences in muscle function between the groups before training. The peak torque/kg on the right side (p<0.01) and left side (p<0.05) and the average power on the right and left sides (p<0.01) showed a significant time effect. Based on the post-hoc analysis, the HCHO group showed a significant increase in average power on both the right and left sides (p<0.05). The HPRO group showed significant increases in the peak torque (p<0.05) and average power on both the right and left sides (p<0.05). However, there was no significant difference in changes in muscle function between the HPRO and HCHO groups after 12 weeks of resistance exercise (Figure 2).
Anabolic/catabolic hormone level changes

There were no significant differences in anabolic/catabolic hormone levels between the groups before training. The growth hormone (p=0.055), testosterone (p<0.001), and testosterone/cortisol (p=0.058) levels showed a trend and significant time effect. There was a difference in cortisol levels between the groups (p<0.05), and a group × time effect was noted for the testosterone (p<0.05), cortisol (p=0.059), and testosterone/cortisol (p<0.05) levels. Based on the post-hoc analysis, the HCHO group showed a significant increase in testosterone (p<0.05) and testosterone/cortisol (p<0.05) levels. There was a difference in the testosterone (p<0.05), cortisol (p=0.059), and testosterone/cortisol (p<0.05) levels between the groups in relation to the amount of change in the anabolic/catabolic hormone levels after 12 weeks of resistance exercise (Table 2).

Blood amino acid level changes

There were no significant differences in the amino acid levels between the groups before training. The essential amino acid (EAA) (p<0.05) level showed a significant time effect. A group × time effect was noted for the EAA (p<0.05) and non-essential amino acid (NEAA) (p<0.01) levels. Based on the post-hoc analysis, the HPRO group showed a significant increase in the EAA and NEAA levels (p<0.01). There was a difference in the EAA (p<0.05) and NEAA (p=0.059) levels between the groups in relation to the amount of change in the amino acid levels after the 12 weeks of resistance exercise (Figure 3).

Creatinine and nitrogen level changes

There were no significant differences in the creatinine and nitrogen levels between the groups before training. The blood nitrogen level showed a significant time effect (p<0.05). However, there were no significant differences in the changes in the creatinine and nitrogen levels between the HPRO and HCHO groups after the 12 weeks of resistance exercise (Table 3).

Discussion

The most important finding in this study is that protein supplementation of carbohydrate intake during the 12-week resistance exercise period increased the testosterone, cortisol, and testosterone/cortisol levels in the HCHO group; moreover, the muscle mass, peak torque, and EAA level in the HPRO group showed more favorable values than those in the HCHO group. Therefore, protein supplementation of carbohydrate intake increased muscle mass and function by increasing the blood EAA levels and stimulating MPS.

It has been reported that resistance exercises positively affect body composition by reducing body fat percentage and fat-free mass [33]; dietary control during resistance exercise further reduces the body fat
percentage; moreover, a low-carbohydrate and high-protein diet and exercise reduce weight by promoting reduction of the body fat percentage and decreasing fat-free mass loss [34]. In this study, there was no difference in body composition, as measured by weight, BMI, and body fat percentage, between the HCHO and HPRO groups after the resistance exercise; all decreased significantly. However, the HCHO group showed a 0.29-kg muscle mass decrease, and the HPRO group showed a 1.67-kg increase, although the difference was not significant.

Figure 2: Changes in muscle function after 12-week resistance training. HCHO: High Carbohydrate intake group; HPRO: High Protein intake group; R: Right side; L: Left side.*Significant change between pre- and post-values in each group based on post-hoc analysis (*p<0.05, **p<0.01).

| Variables       | Group   | Pre     | Post    | Δ (%)   | Time  | Time × Group |
|-----------------|---------|---------|---------|---------|-------|--------------|
| Insulin (mIU/mL)| HCHO    | 6.8 ± 5.2| 9.3 ± 13.3| 132.3 ± 410.1 | 0.477 | 0.912       |
|                 | HPRO    | 3.6 ± 1.7| 5.4 ± 2.5| 57.4 ± 69.8  |       |              |
| HGH (ng/mL)     | HCHO    | 0.9 ± 0.4| 2.4 ± 3.4| 80.6 ± 209.6 | 0.055 | 0.343       |
|                 | HPRO    | 0.5 ± 0.3| 4.9 ± 7.7| 294.1 ± 731.5|       |              |
| Testosterone (ng/mL)| HCHO | 5.4 ± 1.2| 7.0 ± 1.5*| 0.3 ± 0.2  | 0     | 0.038       |
|                 | HPRO    | 6.5 ± 1.4| 7.1 ± 1.8| 0.1 ± 0.18  |       |              |
| Cortisol (mmol/L)| HCHO   | 12.6 ± 3.1| 10.9 ± 2.7| -8.5 ± 31.6 | 0.820 | 0.059       |
|                 | HPRO    | 13.9 ± 3.7| 16.1 ± 3.2| 19.5 ± 24.8#|       |              |
| Testosterone/Cortisol | HCHO | 0.5 ± 0.2| 0.7 ± 0.2*| 0.6 ± 0.7  | 0.058 | 0.011       |
Table 2: Changes in the anabolic and catabolic hormone levels after the 12-week resistance training.

| Variables | Group    | Pre       | Post      | Δ          | Time     | Group   |
|-----------|----------|-----------|-----------|------------|----------|---------|
| Blood Creatine (ng/dL) | HCHO   | 1.1 ± 0.1 | 1.1 ± 0.1 | 0.0 ± 0.1 | 0.076    | 0.787   |
|           | HPRO   | 1.1 ± 0.1 | 1.1 ± 0.1 | -0.0 ± 0.1| 1         |          |
| Blood Nitrogen (mg/dL)   | HCHO  | 13.8 ± 3.3 | 14.4 ± 3.6 | 0.1 ± 0.3 | 0.021    | 0.109   |
|           | HPRO  | 12.0 ± 2.5 | 15.3 ± 3.0 | 0.3 ± 0.2 |          |         |
| Urine Creatine (ng/dL)   | HCHO  | 2.2 ± 1.0 | 1.4 ± 0.7 | -0.3 ± 0.5| 0.203    | 0.345   |
|           | HPRO  | 7.0 ± 13.3 | 1.5 ± 0.6 | -0.3 ± 0.6|          |         |
| Urine Nitrogen (mg/dL)   | HCHO  | 13.5 ± 5.1 | 8.5 ± 3.7 | -0.3 ± 0.5| 0.228    | 0.303   |
|           | HPRO  | 18.9 ± 11.8 | 10.0 ± 2.7 | -0.3 ± 0.6|          |         |

Values are presented as means ± standard errors. *Difference between the pre- and post-12-week resistance training values; p<0.05. #Δ (%) difference between the HCHO and HPRO groups after the 12-week resistance training. HCHO: High-Carbohydrate intake group; HPRO: High-Protein intake group.

Figure 3: Changes in blood amino acid levels after 12-week resistance training. HCHO: High Carbohydrate intake group; HPRO: High Protein intake group; EAA: Essential Amino Acid; NEAA: Nonessential Amino Acid; BCAA: Branched Chain Amino Acid. *Significant change between pre- and post-values in each group based on post-hoc analysis (p<0.01).

Table 3: Changes in the blood and urine amino acid levels after the 12-week resistance training.

Previous studies have shown that medium- to high-intensity resistance exercise at 80% of 1 RM increased muscle mass, hypertrophy, and strength [35]. Moreover, it has been reported that proper protein intake during resistance exercise improves muscle mass and hypertrophy by stimulating muscle protein metabolism and promoting protein synthesis [36]. Williams [37] reported that muscle mass increased more significantly after resistance exercise in a protein-supplemented group than in a non-supplemented group, which is consistent with the results of this study, in which the HPRO group had a greater increase in muscle mass than the HCHO group.

Differences have been observed in the effects of diet on body composition, as shown by an increase in protein in fat-free tissue with use of a protein supplement [33], in addition to reduction in body fat, increase in fat oxidation, increase in metabolic efficiency, and MPS stimulation [38,39]. Conversely, carbohydrate intake after resistance training can attenuate muscle protein breakdown, with no effect on MPS [23]. Although there was no difference in the reduction in weight and body fat percentage based on the differences in dietary intake, the greater increase in muscle mass in the HPRO group might reflect the result of such a mechanism.

The peak torque represents the muscle tension in the dynamic state. This study showed that the peak torque increased on both the right and left sides only in the HPRO group, and that the average power on both the right and left sides increased in both the HCHO and HPRO groups. However, a previous analysis of the increase in muscle strength with protein intake during long-term resistance exercise showed that the protein-supplemented group had an increase in muscle strength in the squat and leg press after 10-week resistance exercise but no difference in muscle strength in the bench press compared with that in the control group [40]. Further, no difference was observed between groups in the increases in muscle strength induced by squats [40]. Moreover, Chromiak et al. [41] reported no significant difference in muscle strength and endurance between amino acid, creatine, whey protein, and carbohydrate supplement intake groups and a carbohydrate intake alone group after 10-week resistance exercise. However, other studies have shown that protein supplementation enhances power performance and knee extensor strength [42,43], and that a higher mixed MPS rate may be related to the increased peak power [44]. Our results also corroborate those of previous studies suggesting that the peak torque increased only in the HPRO group, with inconsistent increases in the muscle mass and blood EAA levels, although the average power increased significantly in both groups.

The response of hormones to resistance exercise has an important role in muscle anabolism and catabolism for MPS. The levels of growth...
hormone, testosterone, cortisol, and insulin increased during and after resistance exercise [45,46]. Growth hormone, testosterone, and insulin have an important role in anabolism, such as the restoration of glycogen and MPS [47,48].

Resistance exercise initially depleted muscle glycogen, and subsequently increased synthesis and promoted replacement of glycogen by increasing insulin sensitivity for up to 48 hours after exercise [49]. A high-intensity workout maintained the increase in insulin for 24 hours after exercise [50], and carbohydrate and protein intake also stimulated insulin release [51]. Although insulin may inhibit protein breakdown and activate the signals that initiate protein synthesis, an increase in the insulin level did not affect protein synthesis or breakdown without an increase in the availability of amino acids [52]. This study found that the insulin levels in the two groups did not significantly increase after resistance exercise, although the HCHO group had higher insulin levels than the HPRO group. It is suggested that the insulin level did not change during the rest time after sustained resistance exercise because the rise in hormone level shortly after exercise stimulated the remodeling of tissues, and the sustained rise in hormone levels after resistance exercise down regulated the receptors [49]. This study did not measure the insulin level shortly after exercise. However, it is thought that the results did not show the synergistic effect of exercise because the blood insulin level was measured during rest time.

Growth hormone is a potential regulator of muscle hypertrophy, and its level increases significantly during and 30 minutes after resistance exercise. High-intensity large-muscle group exercise increases not only the metabolic requirements with changes in the blood acid-base balance but also the secretion of growth hormone [45]. This study showed that the growth hormone levels in both the HCHO and HPRO groups significantly increased after the 12-week resistance exercise (p<0.055). This result shows the enhanced effect of anabolic hormones shortly after sustained resistance exercise, and is thought to be a result of remodeling and adaptation of the muscles during the 12 weeks of exercise.

It has been suggested that testosterone has an important effect on the adaptation to resistance exercises and increases protein synthesis by converting the androgen acceptor of the transcription factor and revitalizing muscle satellite cells [53]. Moreover, testosterone plays a very important role in the adaptation of muscle hypertrophy to resistance exercise by increasing the synthesis of muscle contraction protein [54]. In contrast, this and a previous study [55] found no relationship between circulating testosterone levels and changes in the body composition because the HCHO group had a more significant increase in testosterone level after resistance exercise than the HPRO group.

Accordingly, the testosterone/cortisol levels in the two groups differed. The HCHO group showed an increase in the testosterone/cortisol level, whereas the HPRO group showed a reduction. Such a difference is thought to have been due to the reduction in the blood cortisol level in the HCHO group and an increase in the HPRO group. Cortisol plays a catabolic role by increasing blood fat and amino acid levels through an increase in fat breakdown and reduction of protein synthesis in muscle tissue [56]. It has been reported that the blood cortisol level decreased or did not change after long-term endurance exercise [57], but increased after resistance exercise [58]. An increase in exercise intensity especially affects the restoration of damaged tissues by increasing the cortisol level; however, changes in the cortisol level needed for the restoration of damaged muscles vary on the basis of the reaction to body stress [59]. Baty et al. [60] reported that carbohydrate intake increases the insulin level and formation of muscle protein by reducing breakdown. The HCHO group showed a higher insulin level and a lower cortisol level than the HPRO group because both the maintenance of blood glucose level with a diet rich in carbohydrates and the anabolic effect of insulin reduced protein breakdown. However, no differences between the groups and further increases in the muscle mass in the HCHO group were observed, despite the changes in anabolic hormone responses. Therefore, our results suggest that the changes in the circulating anabolic hormone levels may not correlate with the changes in body composition following resistance training.

With increases in carbohydrate and protein intake, a progressively greater anabolic response with inconstant increases in the insulin response and suppression of protein breakdown occurs [61]. However, most studies indicated that the net protein synthesis is related to the availability of amino acids [51,62,63]. The total EAA and NEAA levels showed a significant difference between the groups; the levels significantly increased in the HPRO group, even without a change in resting levels in the HCHO group. Our results agree with previous findings that a higher protein intake stimulates MPS following resistance exercise [62], and can generate greater muscle mass and strength gains [63].

In conclusion, this study showed that the HPRO group had a greater increase in muscle mass and strength than the HCHO group, although the HCHO group had a greater increase in anabolic hormone levels after the 12-week resistance exercise. These results indicate that MPS was more affected by the availability of amino acids, especially EAA, than by circulating anabolic hormones. These results may indicate that 1.3-g protein intake per kg of body weight is insufficient to increase muscle mass during high-intensity resistance exercise. Therefore, this study suggests that Asians may experience a slightly greater increase in muscle strength and hypertrophy if their diet has less carbohydrate and more protein because their carbohydrate intake is already sufficient. This study only attempted to adjust the ratio of carbohydrate to protein in the diet. Therefore, the results are meaningful as they are easily applicable and highly sustainable for ordinary individuals who are striving to increase muscle mass through exercise.

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