Original Article

New approach for weight reduction by a combination of diet, light resistance exercise and the timing of ingesting a protein supplement

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We have reported that ingesting a meal immediately after exercise increased skeletal muscle accretion and less adipose tissue accumulation in rats employed in a 10 week resistance exercise program. We hypothesized that a possible increase in the resting metabolic rate (RMR) as a result of the larger skeletal muscle mass might be responsible for the less adipose deposition. Therefore, the effect of the timing of a protein supplement after resistance exercise on body composition and the RMR was investigated in 17 slightly overweight men. The subjects participated in a 12-week weight reduction program consisting of mild energy restriction (17% energy intake reduction) and a light resistance exercise using a pair of dumbbells (3–5 kg). The subjects were assigned to two groups. Group S ingested a protein supplement (10 g protein, 7 g carbohydrate, 3.3 g fat and one-third of recommended daily allowance (RDA) of vitamins and minerals) immediately after exercise. Group C did not ingest the supplement. Daily intake of both energy and protein was equal between the two groups and the protein intake met the RDA. After 12 weeks, the bodyweight, skinfold thickness, girth of waist and hip and percentage bodyfat significantly decreased in the both groups, however, no significant differences were observed between the groups. The fat-free mass significantly decreased in C, whereas its decrease in S was not significant. The RMR and post-meal total energy output significantly increased in S, while these variables did not change in C. In addition, the urinary nitrogen excretion tended to increase in C but not in S. These results suggest that the RMR increase observed in S might be associated with an increase in body protein synthesis.

Key words: energy output, exercise, Japanese, protein supplement, resting metabolic rate, weight reduction.

Introduction

It is well recognized that maintaining fat-free mass (FFM) or preventing its decrease is important to successful weight reduction. This is because a decrease in FFM slows the rate of weight reduction as a result of the concomitant decrease in resting metabolic rate (RMR).[1] The major component responsible for a decrease in the RMR is skeletal muscle.[1,2] Therefore, exercise training has been employed to maintain the FFM or to make smaller its decrease during weight reduction.[3]

Respiratory exchange ratio (RER) was observed to be lower for 15 h following a strenuous bout of weight lifting.[4,5] These studies suggest that fat oxidation increased during recovery after resistance exercise. However, there are inconsistencies in the effect of resistance exercise training on fat oxidation.[6–8] The reasons for these discrepancies are not clear although, experimental conditions such as the type of training program could play a role.

We have reported that protein synthesis in the skeletal muscle increased to a greater extent in dogs given a mixture of amino acids and glucose immediately after treadmill running than in dogs given the same nutrients 2 h after the exercise.[9] The study examined the long-term effect of the timing of nutrition provision and demonstrated rats that consumed meals immediately after resistance exercise for 10 weeks obtained higher skeletal muscle accretion and less adipose tissue accumulation than rats who consumed meals 4 h after the exercise.[10] These results led us to hypothesize that a higher RMR as a result of the larger skeletal muscle mass in rats that ingested meals immediately after exercise might be associated with the less accumulation of adipose tissue.

Protein is the predominant component of skeletal muscle, and dietary protein is necessary to synthesize skeletal muscle protein. The administration of amino acids has been shown to increase skeletal muscle protein synthesis.[11] Insulin has been reported to increase protein synthesis in the skeletal muscle.[12] In addition, it has been reported that insulin is necessary to stimulate skeletal muscle synthesis after resistance

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Table 1. Physical characteristics of the subjects at baseline and after the 12-week weight reduction period

	C (*n* = 8)		S (*n* = 9)	
	Baseline	Week 12	Baseline	Week 12
Age (year)	34.0 ± 2.6		33.1 ± 2.3	
Height (cm)	170.1 ± 2.6		170.9 ± 1.9	
Weight (kg)	76.2 ± 4.8	72.1 ± 5.0*	74.9 ± 2.5	70.7 ± 2.8*
Body mass index (kg/m^2)	26.2 ± 1.2	24.8 ± 1.3*	25.6 ± 0.6	24.2 ± 0.7*
Skinfold thickness (mm)				
Tricipital	15.9 ± 1.9	14.0 ± 2.1*	15.1 ± 1.1	11.9 ± 0.9*
Subscapular	21.4 ± 1.8	19.7 ± 1.8	19.5 ± 2.1	18.3 ± 1.7
Total[1]	37.3 ± 3.6	33.7 ± 3.8*	34.6 ± 3.1	30.1 ± 2.5*
Fat%	21.9 ± 1.7	20.2 ± 1.8*	20.6 ± 1.5	18.5 ± 1.2*
Fat mass (kg)	17.1 ± 2.3	15.0 ± 2.3*	15.7 ± 1.7	13.2 ± 1.3*
Fat-free mass (kg)	59.1 ± 2.8	57.1 ± 3.1*	59.3 ± 1.2	57.5 ± 1.8
Girth				
Waist (cm)	87.8 ± 3.1	81.3 ± 3.5*	85.3 ± 1.5	80.5 ± 2.0*
Hip (cm)	97.7 ± 2.6	94.1 ± 2.7*	97.7 ± 1.2	94.6 ± 1.5*
Waist-to-hip ratio	0.90 ± 0.02	0.86 ± 0.02*	0.87 ± 0.02	0.85 ± 0.01*

Values are the means ± SEM. *$P < 0.05$ versus baseline. C, control group, S, supplement group. [1]Total is the sum of the skinfold thicknesses at upper arm and subscapular.

exercise.[13] Furthermore, the administration of amino acids along with glucose has been shown to increase insulin concentration in the plasma[14,15] and stimulate skeletal muscle protein accretion further than the administration of amino acids alone.[16–18]

The present study therefore investigated whether or not the ingestion of a supplement containing protein and carbohydrate immediately after exercise could maintain the FFM and the RMR in slightly overweight men participating in a weight-reduction program which consisted of both mild energy restriction and resistance exercise.

Materials and methods

Subjects and experimental group

Seventeen slightly overweight men (22–47 years of age) participated in a 12-week study. Subjects (standard body weight >10%, body mass index (BMI) >20, fat percentage >20%) were recruited at a college (*n* = 1) and a factory of a pharmaceutical company (*n* = 16). One subject played golf at a driving range once a week and another subject performed resistance exercise twice a week. All other subjects were not involved in regular exercise. The characteristics of the subjects are shown in Table 1. This study was conducted in accordance with the internationally agreed ethical principles for the conduct of medical research. Prior to beginning the study, written informed consent was obtained from each subject.

The subjects were assigned to one of two groups with the means and standard deviations of body compositions which were equal between the groups before the study commenced. Nine subjects assigned to the S group ingested a protein supplement immediately after exercise training and 8 subjects assigned to the C group did not ingest the supplement. The supplement contained 10 g of protein, 7 g carbohydrate, 3.3 g fat, and one-third of the recommended daily allowance (RDA) of vitamins and minerals for Japanese adults.

One subject in the C group was excluded from blood and breath analysis because of an incomplete metabolic measurement at week 12. One subject in the S group was also excluded from the energy expenditure analysis because of an incomplete breath measurement at week 12.

Exercise program

The subjects performed a light resistance exercise training program using a pair of dumbbells (3–5 kg) every day. The exercise training began at 17.00 and continued for about 25 min. The resistance exercise program consisted of 13 types of exercise with 10–15 repetitions including shoulder presses, bent rowing, squatting, twisting, standing dumbbell lifting, butterflies, bent lateral raises, lateral rowing, dumbbell front-curling, one arm dumbbell rowing, dumbbell side-curling, triceps kickbacks, and French presses. The weight of the dumbbell at the start of the study was 3 kg depending on the muscle strength of each subject. The weight of the dumbbell was gradually increased, as the subjects were able to exercise with heavier weights. The final weight of dumbbell was 4 or 5 kg.

Dietary control

The subjects were asked to record their complete food intake for 1 week before the start of the study and throughout the 12-week study period. The daily intake of nutrients was calculated from these records (Table 2). The daily energy intake of the subjects was reduced by 15% compared to their RDA of energy, however, the protein intake met the RDA for Japanese. Supper was provided from Monday to Friday which consisted of the same menu for all subjects on the same day, delivered from a home delivery food service. All alcoholic beverages and food between meals were prohibited except for on weekends and national holidays (5 days). Supper was consumed 1.5 h after the exercise training. Boiled fish paste was added to the supper for the subjects in C to match the daily intake of protein and carbohydrate of the subjects in S. The subjects were allowed to eat freely on the weekends and national holidays. However, they were requested not to overeat. When the subjects ate more than the recommendations during weekends or holidays, their energy intake was reduced accordingly during the subsequent weekdays.

The subjects in both groups took a vitamin supplement (C-Max Multivitamin, Otsuka Pharmaceutical, Tokyo, Japan) per day to prevent any deficiency of vitamins. The vitamin supplement contained vitamin A = 500 IU, vitamin

Table 2. Dietary intake at baseline and during the study

	C (n = 8)		S (n = 9)	
	Baseline	During study	Baseline	During study
Energy (kJ/day)	9680 ± 399	8043 ± 248*	9700 ± 657	8007 ± 205
Protein (g/day)	86.3 ± 4.8	81.5 ± 2.3	87.1 ± 5.6	82.7 ± 2.7
Fat (g/day)	78.8 ± 5.4	65.1 ± 2.4*	80.2 ± 3.8	63.0 ± 3.4*
Carbohydrate (g/day)	282.2 ± 9.1	227.7 ± 6.8*	270.1 ± 22.4	225.7 ± 4.4

Values are the means ± SEM. *P < 0.05 versus baseline.

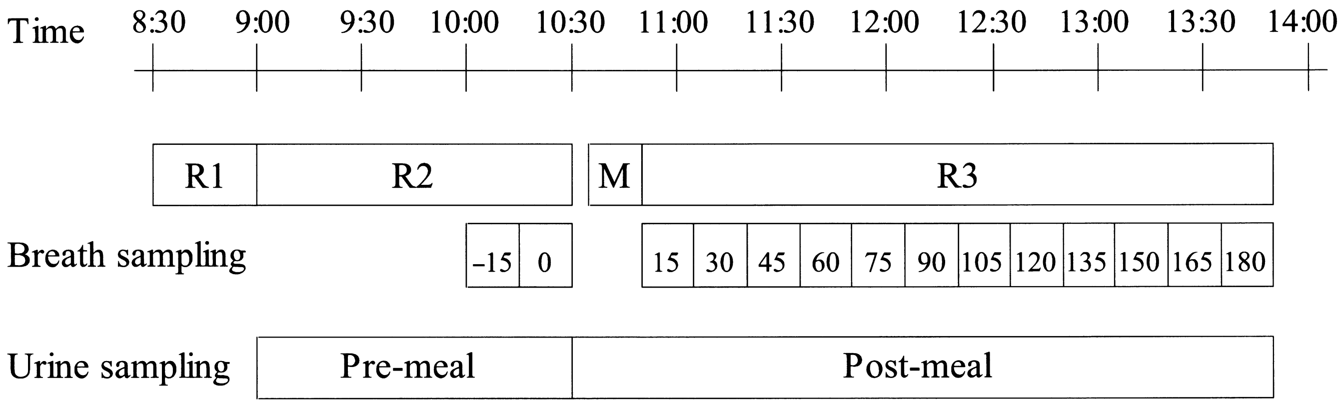

Figure 1. Outline of metabolic measurement protocol. M, ingestion of the test meal; R1, resting in a supine position; R2, rest in the sitting position before ingesting the test meal; R3, resting in a sitting position after ingesting the test meal.

B1 = 1.0 mg, vitamin B2 = 1.4 mg, vitamin B6 = 2.0 mg, vitamin B12 = 6.0 µg, vitamin C = 500 mg, niacin = 17 mg, pantothenic acid = 10 mg, folic acid = 0.4 mg, vitamin D = 25 IU and vitamin E = 4 mg in one tablet.

When the calcium (Ca) and iron (Fe) intake was less than the RDA, according to the weekly food intake evaluation, the subjects were instructed to take a calcium supplement (Ca 300 mg/1 tablet, Nature Made Calcium, Otsuka Pharmaceutical) and an iron supplement (Fe 3 mg/1 tablet, Nature Made Iron, Otsuka Pharmaceutical).

Metabolic measurement

The RMR, post-meal energy output (PEO) and thermic effect of food (TEF) were measured both at baseline and at week 12 as shown in Fig. 1. The measurements were started at 08:30 after an overnight fasting. After lying quietly in a supine position for 30 min (R1), the subjects were asked to empty their bladders, then maintain a sitting position quietly for 90 min (R2). At 60 min of R2, each subject put on a mask to collect their exhaled breath in order to calculate the RMR for 30 min.[14] After collecting the exhaled breath, the subjects ingested a test meal within 15 min. The test meal contained 26.9 g protein, 87.9 g carbohydrate and 18.6 g fat, and the energy was 2621 kJ. The subjects put on the mask again immediately after the ingestion of the test meal, and then the exhaled breath samples were collected every 15 min for 180 min (R3) to calculate both PEO and TEF.[19] The TEF was calculated as the difference between the PEO and RMR. The breath samples were collected in a Douglas bag (Takei Scientific Instruments, Tokyo, Japan) and the concentrations of oxygen and carbon dioxide were analyzed using a mass spectrometer (WSMR-1400, Westron, Tokyo, Japan).

Analyses

The serum samples obtained at both baseline and week 12 were assayed to determine the concentrations of albumin (Alb, Albumin-HRII BCG, Wako Pure Chemical, Osaka, Japan), total protein (TP, total protein-HRII, Wako Pure Chemical) and urea nitrogen (Merck Auto UN, Kanto Chemical, Tokyo, Japan).

Urine was collected as shown in Fig. 1. The urine volume was measured and the nitrogen concentrations were analyzed using the depressor chemiluminescence method (Total Nitrogen Analyzer TN-05, Mitsubishi Chemical Industries, Tokyo, Japan).

Body composition

Height was measured at baseline. The fasting bodyweight was measured to the nearest 0.1 kg and the subscapular and the tricepital skinfold thickness were measured to the nearest 0.1 mm with a Harpenden skin fold caliper (British Indicators, West Sussex, UK) at both baseline and week 12. The body density was calculated using the Nagamine equation based on the skinfold thickness.[20] The relative bodyfat percentage (fat%) was calculated by the Brozek equation.[21] The waist and hip girth were measured to the nearest 1 mm. The fat mass and FFM were calculated from the bodyweight and the fat%.

Statistical analysis

The mean and standard error of the mean (SEM) were reported for all measurements. The difference between S and C at baseline and week 12 were evaluated using the Student's t-test. The paired t-test was used to compare the values between the baseline and week 12. A value of $P < 0.05$ was considered to be significant.

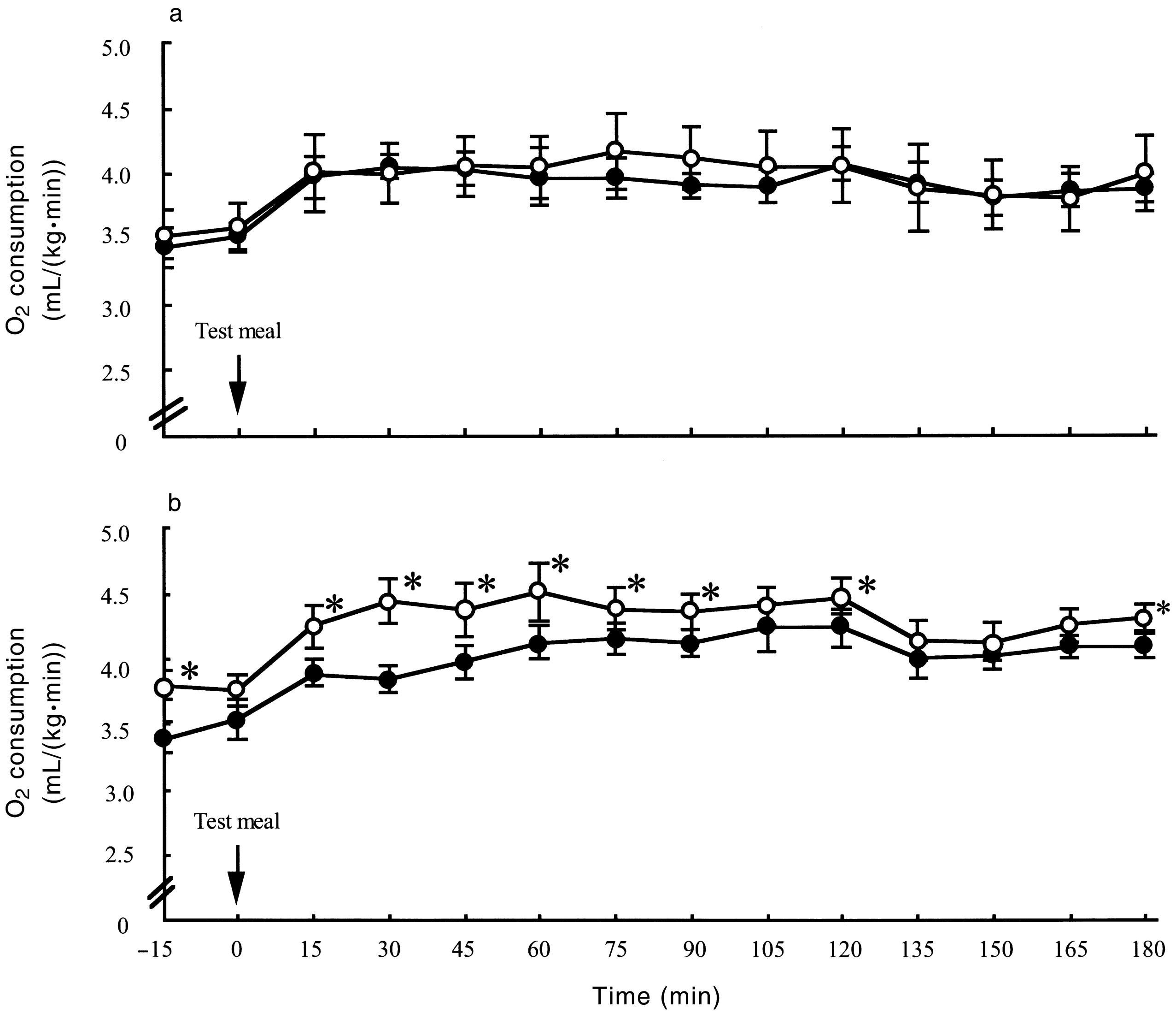

Figure 2. Oxygen consumption during the metabolic measurement at baseline (–●–) and week 12 (–○–). Values are the means ± SEM. *$P < 0.05$ versus baseline. (a) C, control ($n = 7$). (b) S, supplement ($n = 8$).

Results

Food intake

At baseline, the daily energy intake did not differ between S and C (Table 2). In each group, the energy intake was higher than the RDA for Japanese adults. The daily energy intake decreased by 17% in both groups during the study period compared with the baseline, and consisted of about 87% of their RDA. However, because of the large variance in the energy intake in S at the baseline, the decrease was not significant in S ($P = 0.056$). The decrease in energy intake was because of the decrease in the intake of fat and carbohydrate. No difference in the energy intake was found between the groups during the study period. The daily intake of protein did not differ between the groups either at baseline or during the study period. The protein intake did not change during the study period and met the RDA for all subjects.

Body composition

At baseline, no difference was observed between the groups regarding any of the measurements (Table 1). After 12 weeks, the bodyweight ($P < 0.05$), BMI ($P < 0.05$), fat% ($P < 0.05$), and fat mass ($P < 0.05$) decreased significantly in both groups. The FFM decreased significantly in C by 2.1 kg ($P < 0.05$). Although the decrease in FFM in S (1.8 kg) was not statistically significant, the changes were basically similar in both groups. The girth of waist and hip, as well as the ratio of waist to hip decreased significantly ($P < 0.05$) in both groups. The subscapular skinfold thickness did not decrease, however, the tricipital skinfold thickness decreased significantly ($P < 0.05$) in each group. Consequently, the sum of the tricipital and subscapular decreased significantly ($P < 0.05$) in each group. At week 12, no significant difference was observed in any measurements between S and C.

Metabolic measurements

The oxygen consumption during the 3 h metabolic measurement did not differ between the two groups at baseline (Fig. 2). The oxygen consumption increased after ingesting the test meal and sustained high levels until 180 min after the ingestion in both groups at baseline. After the 12-week study, the oxygen consumption increased significantly in S at –15, 15, 30, 45, 60, 75, 90, 120, and 180 min compared to the values of the corresponding time points at baseline. However, no such increase was found in C.

Both groups showed comparable RMR, PEO and TEF at baseline (Table 3). The RMR, which was expressed at the

Table 3. Metabolic measurements at baseline and after the 12-week weight reduction period

	C (*n* = 7)		S (*n* = 8)	
	Baseline	Week 12	Baseline	Week 12
RMR				
(J/(kg × min))	69.1 ± 2.1	69.9 ± 3.5	68.1 ± 2.0	73.6 ± 1.9*
(J/(kg FFM × min))	89.7 ± 1.4	88.5 ± 3.7	84.8 ± 3.4	89.6 ± 2.3
PEO				
(J/(kg × min))	79.6 ± 2.2	80.3 ± 4.5	81.4 ± 2.1	86.6 ± 2.6*
(J/(kg FFM × min))	103.2 ± 1.3	101.6 ± 4.6	101.2 ± 3.0	105.4 ± 2.5*
TEF				
(kJ/(kg × 3 h))	1.9 ± 0.2	1.9 ± 0.3	2.4 ± 0.3	2.4 ± 0.3
(kJ/(kg FFM × 3 h))	2.4 ± 0.3	2.4 ± 0.3	3.0 ± 0.3	2.8 ± 0.3
RER				
Pre-meal	0.87 ± 0.02	0.83 ± 0.03	0.82 ± 0.01	0.80 ± 0.01
Post-meal	0.93 ± 0.00	0.92 ± 0.01	0.91 ± 0.01	0.91 ± 0.01

Values are the means ± SEM. *$P < 0.05$ versus baseline. RMR, resting metabolic rate; FFM, fat-free mass; PEO, postprandial total energy output; TEF, thermic effect of food; RER, respiratory exchange ratio.

Table 4. Serum total protein, albumin and BUN at baseline and after the 12-week weight reduction period

	C (*n* = 7)		S (*n* = 9)	
	Baseline	Week 12	Baseline	Week 12
Total protein (g/L)	69 ± 1	70 ± 1	70 ± 1	70 ± 1
Albumin (g/L)	43 ± 2	43 ± 2	46 ± 1	45 ± 1
Urea nitrogen (mmol/L)	5.0 ± 0.1	5.4 ± 0.2	5.5 ± 0.2	5.6 ± 0.3

Values are the means ± SEM; BUN, blood urea nitrogen.

Table 5. Urinary nitrogen excretion at baseline and after the 12-week weight reduction period

	C (*n* = 7)		S (*n* = 9)	
	Baseline	Week 12	Baseline	Week 12
Urinary nitrogen excretion (mmol/day)				
Pre-meal	870 ± 90	1010 ± 90	820 ± 70	890 ± 30
Post-meal	960 ± 70	980 ± 90	870 ± 30	910 ± 50

Values are the means ± SEM.

bodyweight basis, increased significantly in S ($P < 0.05$) and the RMR based on the FFM also tended to increase in S ($P = 0.07$). In contrast, the RMR showed no change in C. The PEO increased significantly after the 12-week study in S ($P < 0.05$), however, no significant change was observed in C.

The TEF did not change after the 12-week study in either group. The pre-meal and post-meal non-protein respiratory exchange ratio (RER) did not differ between the groups at baseline, and did not change significantly in both groups after 12 weeks.

Serum and urine parameters

As shown in Table 4, the baseline serum levels of total protein, albumin and urea nitrogen did not differ between the groups. After the 12-week study, the concentration of serum urea nitrogen tended to increase in C ($P = 0.08$), whereas no change was observed in S. The serum concentration of nitrogen during both the pre-meal and post-meal periods did not differ between the groups at baseline (Table 5). At 12 weeks, the urinary nitrogen excretion during the pre-meal period tended to increase in C ($P = 0.06$). However, no significant change was found in S (Table 5).

Discussion

As the RMR accounts for 65–70% of the daily energy expenditure, even a small change in the RMR can be a determinant of the change in bodyweight and body composition.[22] An acute bout of resistance exercise has been reported to increase the RMR.[4,5,23–27] Trained subjects have also been reported to have a higher RMR compared to untrained subjects.[28,29] However, intervention studies have not always confirmed this phenomenon. Resistance exercise training increased the RMR in some studies,[8,30,31] whereas the RMR did not change in other studies.[7,32] These controversial results appear to be related to the age of the subjects. Studies have shown that elderly subjects are apparently more susceptible to an increase in the RMR by resistance exercise training[8,30,31] than young subjects.[7,32] The present study showed the RMR expressed per kilogram bodyweight significantly increased in the subjects who took the supplement immediately after exercise. However, the subjects who did not take the supplement showed no change after the 12-week study. This study is the first study to examine the implications of the ingesting timing of food in relation to resistance exercise training. According to this result, nutritional values of the nutrition regimen used in the present study might be enhanced by the ingestion timing, and this might be associated with any differences between the current results and previous ones.

In the present study, however, the FFM was not different between S and C. Therefore, the increased RMR observed in S appears not to be because of an increase in the FFM. One of the plausible mechanisms associated with the increase in the RMR in S might be an increased protein turnover. The RMR has been reported to be a function of protein turnover.[33] We previously showed that the skeletal muscle protein synthesis rate to be higher when a mixture of amino acids and glucose was administered immediately after exercise compared with 2 h after ingestion.[9] Although it is not clear as to how long this elevated protein synthesis rate (which is a component of protein turnover) continued, the turnover rate might be higher in the subjects in S than the subjects in C for some duration of time after exercise. Thus, this possible increase may have resulted in a higher RMR in S than in C. The entire mechanisms are not clear, however, it appears that taking the protein supplement immediately after resistance exercise may increase the RMR without increasing the FFM.

It has shown that, even if aerobic exercise training is performed, the FFM decreased during a weight reduction period when the energy balance was negative.[2,3] The energy intake was reduced by >50% in those studies. In the present study, the energy intake was only restricted by 17%. Although this restriction was milder than the previous studies,[2,3] the resistance exercise effect which has been known to increase the FFM could not surpass the FFM reduction associated with a negative energy balance in the current study. However, it may be interesting to note that the decrease in the FFM was statistically significant in C, while the decrease was not statistically significant in S. Even the degree of the decrease did not differ between C and S very much. The serum urea nitrogen concentration tended to increase in C after the 12-week study, whereas it showed no change in S. In addition, the urinary excretion of nitrogen tended to increase in C after the study, whereas it did not change in S. These results suggest that a larger amount of amino acids might have been metabolized in C than in S, thus resulting in a larger amount of nitrogen being excreted in C than in S. Therefore, the ingestion of a protein supplement immediately after resistance exercise may stimulate protein retention in the body, thus reducing a decrease in the FFM during the current study period.

The TEF was not influenced in either group after the study. An acute bout of exercise preceding a meal has been shown to enhance the TEF.[34–39] Trained subjects have been reported to have a higher TEF than untrained subjects.[29] Although the TEF did not change after the 12-week study, the PEO for 3 h after the meal increased significantly in S, whereas it did not change in C. As the RMR increased significantly in S, the elevated PEO after the 12 weeks observed in S was accounted for the elevated baseline of the metabolic rate.

In the present study, RER did not change after the 12-week study in each group. There are inconsistencies in the effect of resistance exercise training on fat oxidation.[4–8] The reason for these discrepancies are not clear although, experimental conditions such as the type of training program could play a role.

The protein requirement has been reported to increase when the energy intake is restricted.[40,41] In the present study, the energy intake was restricted to 87% of the RDA. However, the serum albumin and the total protein concentrations did not decrease after the study period in either group. These results indicate that the protein intake was thus sufficient in the present study which lasted for 12 weeks.

There are several limitations that should be considered when examining the results of the study. The small number of participants in both S and C groups greatly reduced the power of the statistical analyses decreasing the potential of obtaining statistical significance. It is also not enough to estimate the impact of potential confounders on the findings by the same reason. We have not examined the exercise type and duration, although the dumbbell exercise seems to have effects of both resistance and aerobic exercise. Therefore, further study is needed to clarify the importance of ingestion timing of food during weight control.

In conclusion, the RMR and PEO increased significantly in the subjects who ingested a protein supplement immediately after resistance exercise, whereas the subjects who did not ingest this supplement showed no change. As the FFM did not differ significantly between the groups, ingesting the protein supplement immediately after exercise appears to influence body protein metabolism without changing its mass during a weight reduction program consisting of food restriction and resistance exercise.

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