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Effect of Egg White Protein Supplementation Prior to Acute Resistance Training on Muscle Damage Indices in Untrained Japanese Men

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A B S T R A C T

The purpose of this study was to assess the effects of egg white (E) protein supplementation on the muscle damage indices and muscular soreness after acute resistance training (RT) compared with soy (S) or no protein supplementation (C). In this cross-over study, six healthy untrained men completed three RT trials. Participants were asked to consume a meat-free diet and refrain from high-intensity activities during all trial periods. On the day of RT, participants ingested one of three test beverages containing water only or water containing either 20 g of E or S protein 1.5 hours after breakfast, then performed 60 minutes of RT. Blood was drawn at baseline, before, immediately after, and 30 minutes after RT to assess blood glucose, lactate, insulin, growth hormone (GH), creatine kinase activity (CK) and cortisol levels. Urinary 3-methylhistidine (3-MetHis), urea nitrogen (UN), and creatinine (CRE) were measured using 24-h urine samples, and muscular soreness was measured by a visual analog scale. The daily protein intake was approximately 0.8 g/kg body weight in all three groups. Each lactate, GH, CK, cortisol, 3-MetHis, or muscular soreness increased significantly after RT, with no significant differences between the three groups. The UN was significantly higher in the E and S groups compared to the C group. The RT exercise protocol successfully induced blood biochemical changes, muscle damage or muscle soreness in all three groups with no significant differences, and pre-exercise protein supplementation taken in excess may accelerate protein catabolism.

Key words: *3-methylhistidine, urea nitrogen, muscle soreness.*

Introduction

For athletes, the most important issue is how to induce muscle hypertrophy than concerning their health. Evans (1992) proposed that exercise-induced muscle damage or muscle protein breakdown (MPB) may be the primary stimulus for muscle hypertrophy. Furthermore, Phillips, Hartman, and Wilkinson (2005) used established models of muscle amino acid (AA) turnover to examine how protein source (milk versus soy) acutely affects the processes of muscle protein synthesis (MPS) and MPB after resistance exercise training (RT). The authors also showed that even if balanced quantities of total protein and energy were consumed, milk proteins were more effective in stimulating AA uptake and net protein deposition in skeletal muscle after RT than hydrolyzed soy proteins, suggesting that some kinds of protein and AA supplements decrease MPB while concurrently increase MPS (Phillips et al., 2005; Moore et al., 2009).

On the other hand, there are many reports to study the impact of protein supplementation on RT and nutritional interventions such as whey hydrolysate, soy protein or casein (Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009), essential amino acid (EAA, Bird, Tarpenning, & Marino, 2006) or branched-chain amino acids (BCAAs, Howatson et al., 2012). However, there are little available data regarding the effects of egg white protein supplementation on post-exercise MPS or MPB. Egg white protein is also an ordinary, high-protein, low-fat food, and like soy protein, has the highest AA score (100%) (Gilani, 2012). Moore et al. (2009) reported that post-exercise ingesting 20 g of intact whole egg protein was sufficient to maximally stimulate MPS, and AA availability was the main factor driving muscle anabolism associated with feeding after RT. Although it is necessary to

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just carry out protein balance to muscular hypertrophy, there was few available data whether pre-exercise dietary protein supplementation increases post-exercise MPS or MPB.

RT fundamentally stimulates the process of MPS which was depend on exercise-induced increases in endogenous anabolic hormones, such as growth hormone (GH) and insulin-like growth factors (Ghanbari-Niaki, Nabatchian, & Hedayati, 2007; Hoffman et al., 2008; West & Phillips, 2012). In addition, urinary nitrogen (UN) sometimes used as an index of protein intake (Young, El-Khoury, Raguso, Forslund, & Hambraeus, 2000). Furthermore, a perceived muscle discomfort and soreness (MS), perceived fatigue or urinary 3-methylhistidine (3-MetHis) sometimes used as an index of MPB (Evans, 1992; Lukaski, Mendez, Buskirk, & Cohn, 1981).

The aim of this study was to estimate the effect of pre-exercise egg white protein on bloody and urinary biochemical changes after RT. Our hypothesis was that pre-exercise egg white supplementation, as well as soy protein which was previously reported to be effective in MPS, would produce significant effects on MPS but decrease MPB compared with no protein supplementation. We have used cortisol and GH as indicators of MPS, urinary UN excretion as an index of protein intake, and urinary 3-MetHis and serum creatine kinase (CK) activity as indicators of MPB after RT.

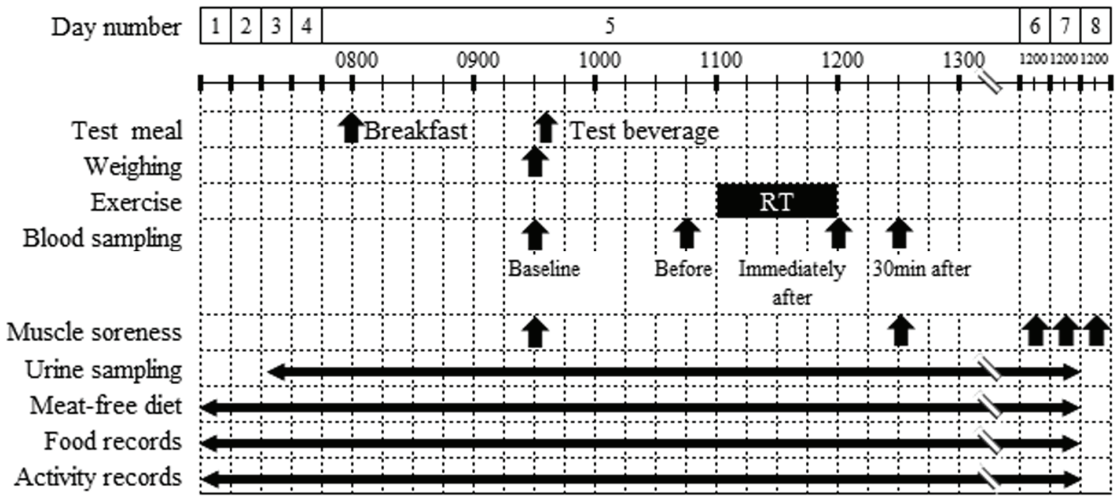
Materials and Metods

Participants

Six healthy male university students with no allergic to egg white or soy, and who did not exercise on a regular basis at least four months prior to study, volunteered to participate in this study. The mean (±SE) age, height, and body mass weight (BW) were 21.2 (±0.3) years, 173.6 (±2.8) cm, and 62.7 (±2.8) kg, respectively. All participants voluntarily provided written informed consent prior to study initiation after a full explanation of all procedures and possible risks of the study. This study was approved by the Human Research Ethics Committee of Tokyo University of Agriculture (ID: 0802, June 2008).

Experimental Design

This cross-over study was based on eight-day testing periods separated by intervals of at least seven days. Each testing period began on Day-1 and ended the meat-free diet consisting of grains, beans, and milk, and 24- hour urine sample collection on Day-8 (Figure 1). Participants were allocated into one of three groups; egg white protein (E), soy protein (S), and mineral water control (C) group with no additive, and all were carried out this study protocol three times, and asked not to change their lifestyle behaviors.



RT: resistance training. Each participant (n=6) followed this protocol and consumed one of the three test beverages. A bout of RT exercise consisted of approximately 10 minutes of warm-up, 40 minutes of RT, and 10 minutes of cool-down exercise. After a seven-day washout period, participants crossed-over into the remaining condition. Each participant performed the experimental protocol three times.

Figure 1. Experimental protocol

On Day-5 RT loading day, participants arrived at the laboratory at 8:00 AM, and had a breakfast consisting of a rice ball (energy, 355 kcal; protein, 6.7 g; carbohydrate, 78.1 g). At 9:30 AM, after baseline blood sample collection and perceived MS measurements, one of the three test beverages was ingested. At 11:00 AM, participants started a RT exercise protocol. Blood samples were collected additional three times (before, immediately after, and 30-minutes after RT), perceived MS was four more times at 30-minutes, 24-hour, 48-hour, and 72-hour after RT, and a 24-hour urine sample was for five days from Day-3 to Day-8.

Test Beverages

Each test protein beverages for the E and S group contained 20 g of protein per package (Table 1), and were prepared iso-energetically and donated by Kewpie Corporation, Tokyo, Japan. Each supplement was delivered as a dry powder in sealed packages with a number code, and was stored in a refrigerator until use. Supplements were reconstituted and dissolved in 200-mL of mineral water prior to intake.

Table 1. Nutrient profiles of protein supplements

	E group	S group
Composition **(26 g/package)**		
Dried egg-white (g)	24	0
Isolated soy protein (g)	0	23.3
Powdered oil (g)	0.8	0.8
Dextrin (g)	1.2	1.9
Nutritional profile **(26 g/package)**		
Energy (kcal)	98	95
Protein (g)	20	20
EAA (mg)	10,240	8,860
BCAA (mg)	4,560	3,940
Amino acid score	100	100

Note. E: egg white protein group; S: soy protein group; EAA: essential amino acids; BCAA: branched chain amino acids. Energy (kcal), protein (g) and amino acids (mg) were calculated based on the Standard Tables of Food Composition in Japan 2010: Amino acid composition of foods.

Resistance Exercise

Two weeks before the experimental protocol, one-repetition maximum (1-RM) strength and preliminary measurements were undertaken. After a standardized warm-up exercise, 1-RM estimates were determined by using multiple-RM tests (Bachle, Earle, & Wathen, 2000) with standard gym equipment (Senoh, Chiba, Japan) such as seated row, fly, leg extension, and leg press. After equipment familiarization, each participant performed five or less loading sets during which the weight stack was adjusted to determine the amount of weight the participant could lift 5-12 times repeatedly. Then, 1-RM was predicted using the determined amount of weight and number of repetitions.

On the Day-5, participants completed warm-up sets, and the RT bout three sets of 10 repetitions at ~80% 1-RM with one min rest between sets and two minutes between each RT exercise. Test protocol was concluded with stretching (10 minutes). Verbal encouragement was consistently given during all attempts.

Rating of Perceived Fatigue and Muscle Soreness

Participants sat on a chair and rated their perceived fatigue and MS at four sites: anterior thigh, posterior thigh, chest, and back using a visual analogue scale which consisted of a line from 0-mm (no fatigue/pain) to 100-mm (unbearable fatigue/pain). The scores at the four sites were summed to obtain a total perceived MS score. Data were expressed in centimeters.

Blood Biochemistry and Urine Analysis

Lactate levels in whole blood were measured with Lactate Pro (Arkray, Inc., Shiga, Japan). A tube containing EDTA was used to collect blood for analyses of red blood cell (RBC) counts, hemoglobin and hematocrit levels, a tube containing sodium fluoride was glucose, and a tube with no additives was triglycerides, total protein, albumin, immunoreactive insulin (IRI), GH, cortisol, and CK, respectively. Blood analyses except lactate were performed by Medical Laboratory Systems (Kanagawa, Japan) in the same run or same assay plate.

A urine sample was collected each day for 24-hours beginning at 7:00 AM one day and ending at 7:00 AM the next day using a portable device (Urine Mate P, Sumitomo Bakelite, Tokyo, Japan). Participants could easily transport this device that could collect one-fiftieth volume from each urine excretion, and 24-hours urine volume was estimated by multiplying the sample quantity of the portable device by 50. Daily urinary levels of 3-MetHis, UN, and creatinine (CRE) were measured by Medical Laboratory Systems (Kanagawa, Japan). Furthermore, the area under the curve (AUC) of 3-MetHis or UN for three days after RT loading was calculated.

Dietary intakes and Activity Records

Participants completed a self-reported weighed food record during each trial to assess compliance with the meat-free diet and changes in daily food intake between trials. They were given verbal and written instructions for food recording before initiation of the first trial. Daily energy and nutrient intake were calculated based on the Standard Tables of Food Composition in Japan 2010 by a registered dietitian. Physical activity levels between trials were also determined using dietary analysis software by a total MET-hour score for that day (Excel Eiyo-kun FFQg ver. 2.5, Kenpakusha, Japan).

Statistics

All data were expressed as mean±SE. A one-factor repeated-measured ANOVA was used to characterize protein types in physical activity level, BW, total weight lifted, dietary intakes, AUC 3-MetHis or AUC UN for 3 days, respectively. Blood biochemical parameters, urinary daily 3-MetHis and UN, and MS were analyzed using a two-factor repeated measures analysis of variance (ANOVA) with within–subjects factors for protein type treatment (egg, soy and control) and time. In cases in which significant main effects or interactions were present, a post hoc analysis was conducted by using Bonferroni adjustments. The α level for significance was set at $p < 0.05$. All data were analyzed using IBM SPSS Statistics 20.0 for Windows (IBM Japan, Tokyo, Japan).

Results

Participant Characteristics

There was no significant difference between the three groups in physical activity levels, BW, total weight lifted on Day-5 and dietary intakes (Table 2). Most of the participants had a dietary protein intake of approximately 0.8 g/kg BW/day. During the experimental periods, participants consumed 62.0% of dietary protein from grain, 16.3% from soy beans, 10.9% from whole egg, 9.1% from milk products, 1.0% from meat, and 0.7% from fish. The volume of mineral water intake during RT was not different between the three groups.

Table 2. Participant characteristics

Characteristics		E	S	C	*P value*[a]
Body weight[b]	(kg)	62.08 ± 3.11	61.94 ± 2.96	62.63 ± 3.34	0.411
Total weight lifted[c]	(kg)	8670 ± 416	8316 ± 399	8505 ± 309	0.553
Physical activity level[d]		1.75 ± 0.09	1.71 ± 0.10	1.78 ± 0.10	0.315
Energy and nutrient intake[d]					
Energy	(kcal/day)	1961 ± 112	1941 ± 105	1849 ± 60	0.375
	(kcal/kg BW/day)	32.2 ± 2.9	32.0 ± 2.9	30.1 ± 2.3	0.502
Protein	(g/day)	52.0 ± 2.9	50.4 ± 2.8	47.7 ± 1.3	0.164
	(g/kg BW/day)	0.85 ± 0.08	0.83 ± 0.07	0.77 ± 0.04	0.090
Fat	(g/day)	47.8 ± 6.4	48.0 ± 5.8	44.5 ± 5.3	0.613
	(g/kg BW/day)	0.79 ± 0.11	0.79 ± 0.11	0.73 ± 0.10	0.529
Carbohydrate	(g/day)	315.4 ± 15.8	308.1 ± 16.8	304.2 ± 9.7	0.713
	(g/kg BW/day)	5.19 ± 0.47	5.08 ± 0.47	4.95 ± 0.40	0.546

Note. Values are expressed as mean±SE (n=6).
E: egg white protein group; S: soy protein group; C: control group (no protein group).
[a]One-factor repeated measures ANOVA
[b]Body weight was at baseline on Day-5.
[c]Participants lifted this total mean weight during RT.
[d]Physical activity level, and energy and nutrient intake were for seven days.

Table 3. Changes in blood biochemical parameters

	Group	Baseline	Before	Immediately after	30 min after	ANOVA *(P value)*[a] G	T	G x T
RBC	E	473 ± 10	465 ± 13	509 ± 15	466 ± 13			
(10^4/μL)	S	479 ± 14	475 ± 11	507 ± 11	475 ± 14	0.191	<0.001	0.346
	C	470 ± 9	465 ± 8	497 ± 13	460 ± 11			
Hb	E	14.2 ± 0.3	14.0 ± 0.4	15.0 ± 0.4	14.0 ± 0.4			
(g/dL)	S	14.3 ± 0.4	14.3 ± 0.4	15.1 ± 0.4	14.2 ± 0.4	0.097	<0.001	0.850
	C	14.0 ± 0.2	14.0 ± 0.2	14.8 ± 0.3	13.7 ± 0.3			
Ht	E	43.5 ± 0.9	42.5 ± 1.0	47.2 ± 1.5	42.7 ± 1.3			
(%)	S	43.7 ± 1.3	43.3 ± 1.0	47.0 ± 1.2	43.3 ± 1.4	0.298	<0.001	0.361
	C	43.0 ± 0.6	42.7 ± 0.8	46.0 ± 1.2	42.2 ± 1.0			
TP	E	6.9 ± 0.1	6.8 ± 0.1	7.8 ± 0.1	6.9 ± 0.1			
(g/dL)	S	7.0 ± 0.2	7.1 ± 0.2	7.8 ± 0.2	7.2 ± 0.3	0.160	<0.001	0.381
	C	6.8 ± 0.2	6.8 ± 0.2	7.6 ± 0.2	6.9 ± 0.2			
Alb	E	4.3 ± 0.1	4.2 ± 0.1	4.9 ± 0.1	4.4 ± 0.1			
(IU/L)	S	4.4 ± 0.2	4.6 ± 0.2	5.0 ± 0.2	4.6 ± 0.2	0.581	<0.001	0.139
	C	4.4 ± 0.1	4.4 ± 0.1	4.9 ± 0.1	4.5 ± 0.1			
Glu	E	89.8 ± 5.0	75.5 ± 4.4	83.5 ± 2.0	84.5 ± 3.3			
(mg/dL)	S	94.8 ± 6.6	73.7 ± 5.9	83.8 ± 3.2	80.7 ± 2.0	0.881	0.037	0.302
	C	95.5 ± 10.2	83.2 ± 2.7	79.2 ± 2.4	81.0 ± 3.5			
TG	E	56.8 ± 11.2	53.3 ± 10.8	57.7 ± 11.6	50.3 ± 10.3			
(mg/dL)	S	72.3 ± 23.5	54.0 ± 13.1	58.0 ± 12.4	51.3 ± 10.5	0.675	0.232	0.342
	C	62.8 ± 19.7	48.8 ± 10.0	48.8 ± 7.2	43.2 ± 5.9			
Lactate	E	1.3 ± 0.1	1.1 ± 0.1	8.6 ± 1.7	2.4 ± 0.6			
(mmol/L)	S	1.1 ± 0.1	1.0 ± 0.1	7.9 ± 1.0	3.0 ± 0.8	0.863	<0.001	0.524
	C	1.3 ± 0.2	1.0 ± 0.1	7.7 ± 1.3	2.7 ± 0.8			
CK	E	122.5 ± 20.6	121.8 ± 19.3	148.0 ± 24.0	141.8 ± 24.7			
(IU/L)	S	113.5 ± 7.8	114.8 ± 7.9	138.0 ± 7.4	136.0 ± 8.2	0.107	<0.001	0.804
	C	163.3 ± 34.7	170.7 ± 38.9	188.2 ± 34.3	179.8 ± 32.5			
IRI	E	10.7 ± 1.6	8.6 ± 0.6	8.1 ± 0.3	7.6 ± 0.3			
(μU/dL)	S	11.8 ± 2.7	9.6 ± 1.0	7.6 ± 0.4	7.8 ± 0.4	0.367	0.191	0.478
	C	18.0 ± 7.9	8.8 ± 0.4	8.1 ± 0.4	7.7 ± 0.2			
GH	E	0.2 ± 0.0	3.8 ± 2.7	19.1 ± 4.2	6.6 ± 2.0			
(ng/mL)	S	0.4 ± 0.2	0.6 ± 0.3	20.9 ± 2.2	8.9 ± 1.9	0.611	0.006	0.462
	C	0.2 ± 0.1	2.1 ± 1.2	27.6 ± 9.4	8.0 ± 0.9			
Cortisol	E	13.9 ± 1.7	10.4 ± 1.4	19.7 ± 2.3	15.4 ± 1.5			
(μg/dL)	S	16.3 ± 2.0	15.6 ± 2.9	20.0 ± 2.8	20.2 ± 3.0	0.197	0.005	0.140
	C	17.7 ± 0.8	13.6 ± 1.3	20.3 ± 2.8	17.3 ± 2.7			

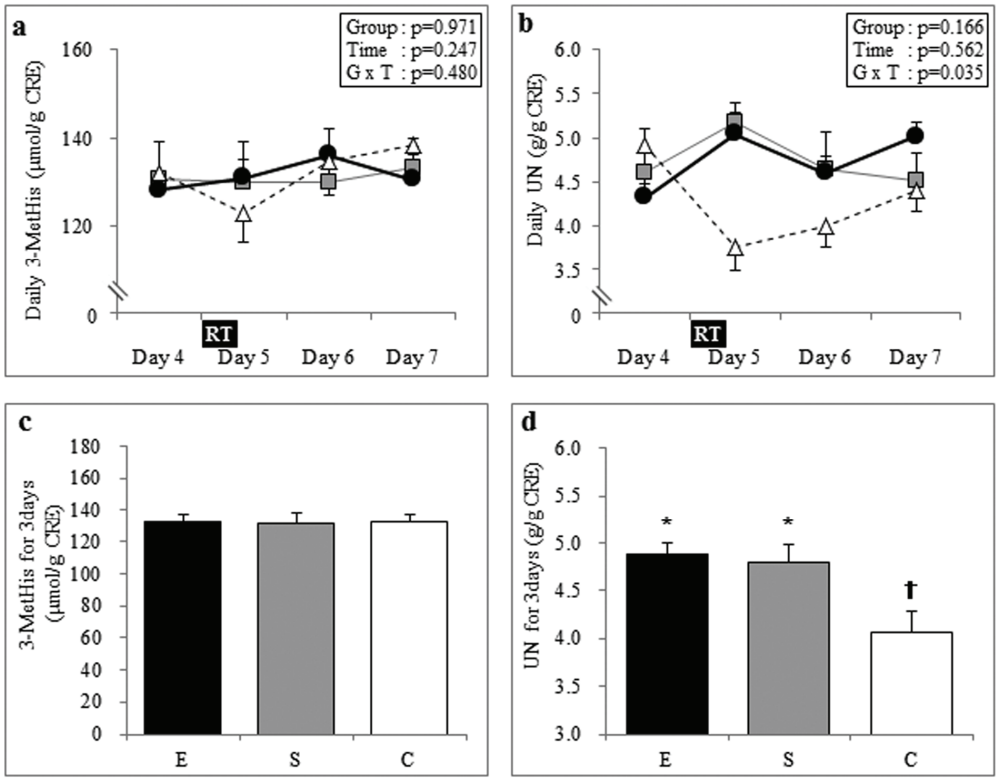
Note. Values are expressed as mean±SE (n=6). E: egg white protein group; S: soy protein group; C: control group (no protein). RBC: red blood cell; Hb: hemoglobin; Ht: hematocrit; Glu: blood glucose; TG: triglyceride; TP: total protein; Alb: albumin; IRI: immunoreactive insulin; GH: growth hormone; CK: creatine kinase activity; G: protein group; T: time; G x T: interaction effect of protein type x time.
[a]Two-factor repeated measures ANOVA was used for the analysis.

Blood Biochemical Parameters

For all blood biochemical parameters, there was no significant main effect of protein type or interaction effect of protein type with time (Table 3). However, there was a significant main effect of time in RBC, hemoglobin, hematocrit, total protein, albumin, glucose, lactate, CK, GH, and cortisol levels. Increases in lactate, CK, GH, and cortisol levels immediately after RT were higher than increases in RBC, hemoglobin, hematocrit, total protein, and albumin. Lactate levels showed an 8-fold increase immediately after RT compared to before, and then decreased to a 2.7-fold increase at 30-minutes after RT ($p=0.013$ and $p=0.007$, respectively). CK increased significantly just after RT and remained high at 30-minutes after RT compared to before ($p=0.003$ and $p=0.020$, respectively). GH and cortisol levels significantly increased immediately after RT and thereafter, gradually decreased 30-minutes after RT. However, there was no significant main effect of time in TG and IRI.

Urinary 3-MetHis and UN Response

Urinalyses were performed except one who failed to collect a urine sample. There were no main effects of protein type and time, or interaction effects of 3-MetHis (Figure 2a), and there was no significant difference in AUC of 3-MedHis by protein type (Figure 2c). In contrast, UN showed a significant interaction effect (Figure 2b). Bonferroni post-hoc test revealed that AUC of UN was significantly higher in both E and S groups compared to C group (Figure 2d; E vs. C group: $p=0.025$, S vs. C group: $p=0.044$).



a: Daily 24-hour urinary excretion values for 3-MetHis (μmoles/g CRE). b: Daily 24-hour urinary excretion values for UN (g/g CRE). c: AUC of the 3-MetHis concentrations for three days. d: AUC of the UN concentrations for three days. Data are expressed as mean±SE (n=5).

E: Egg white protein group, S: Soy protein group, C: Control group.

3-MetHis: 3-methylhistidine, UN: urea nitrogen; CRE: creatinine; RT: resistance training.

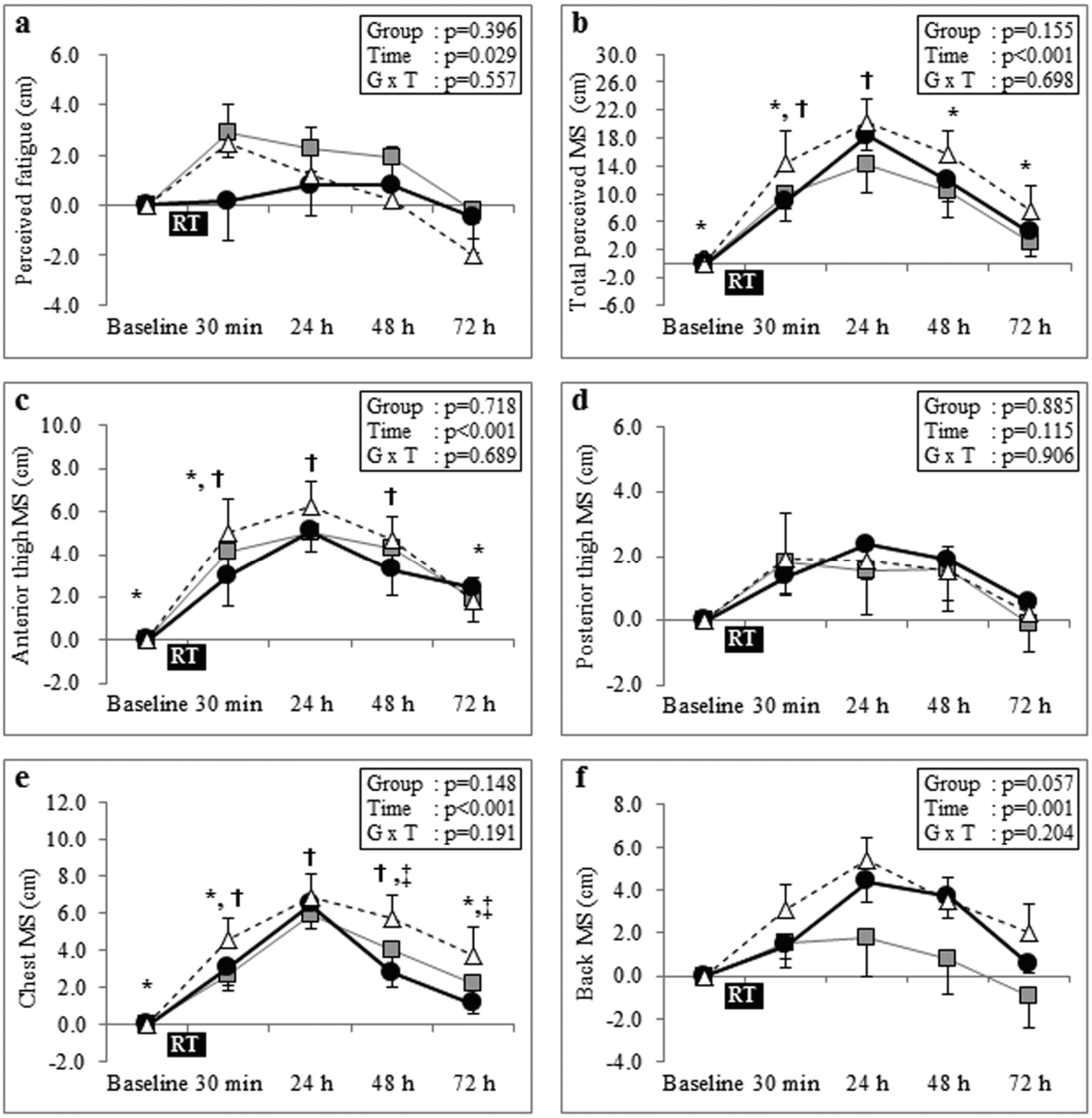
G x T: interaction effect of protein type x time by two-factor repeated measures ANOVA.

A two-factor repeated measures of variance was used for daily 3-MetHis (a) or daily UN (b) analysis, and a one-factor repeated measures of variance (protein group) was used in AUC analysis for 3-MetHis (c) or UN (d) to detect differences. *,† Different letters indicate significant differences ($p<0.05$).

Figure 2. Urinary 3-MetHis and UN excretion following RT loading

Perceived Fatigue and Muscle Soreness

Neither a significant main effect of protein group nor an interaction effect was found in perceived fatigue or all MS scores. A significant main effect of time was observed for perceived fatigue scores (Figure 3a, $p=0.029$). Perceived fatigue scores increased 30-minutes after RT, and then decreased gradually until 72-hours after. Perceived MS scores in the anterior thigh (Figure 3c), chest (Figure 3e), and back (Figure 3f) except for the posterior thigh (Figure 3d), all showed a significant main effect of time. Accordingly, total perceived MS scores showed a main effect of time (Figure 3b). The Bonferroni post-hoc test revealed that anterior thigh, chest, and total MS scores increased 30-minutes after RT and significantly increased 24-hours post-exercise compared with the baseline, respectively. These scores were the highest in the C group, but not significant.



a: Perceived fatigue. b: Total perceived MS. c: Perceived MS in the anterior thigh. d: Perceived MS in the posterior thigh. e: Perceived MS in the chest. f: Perceived MS in the back. Data are expressed as mean±SE scored by VAS (n=6).
E: Egg white group, S: Soy protein group, C: Control group.
RT: resistance training; MS: perceived muscle soreness; G x T: interaction effect of the protein type x time. A two-factor repeated measures of variance was used for perceived fatigue and various MS analysis. As a significant main effect of the time was present, post hoc analysis was conducted by using a Bonferroni adjustment. *,†,‡ Different letters indicate significant differences ($p < 0.05$)

Figure 3. Changes in perceived fatigue and muscle soreness scores following RT loading

Discusion

To our knowledge, this is the first study to investigate the effects of pre-RT supplementation of E protein on muscle damage induced by acute RT exercise in untrained young men. Our protocol successfully caused blood biochemical changes and muscle damage with time, but no significant differences was found between the three groups. Furthermore, urinary UN excretion increased significantly in protein supplementation groups compared to the C group.

In our study, participants ingested 20 g of E or S protein. Moore et al. (2009) examined the ingestion of 0, 5, 10, 20, or 40 g whole egg protein following RT on MPS, and reported that ingestion of 20 g intact protein (~8.6 g EAAs) maximally stimulated MPS. And also dietary protein consumed after RT in excess of the rate at which it could be incorporated into tissue protein stimulated irreversible oxidation. Furthermore, Børsheim, Tipton,

Wolf, and Wolfe (2002) showed that post-exercise stimulation of MPS almost twice as greater after ingestion of 6 g compared with only 3 g EAAs. In our present study, 20 g of E and S protein contained 10.2 g and 8.9 g EAA, respectively. Even if MPS is stimulated with 20 g protein intakes (Moore et al., 2009; Børsheim et al., 2002), participants in our study had sufficient levels of protein or EAA to stimulate MPS.

In addition, hemoglobin, hematocrit, TP, and albumin levels increased significantly immediately after RT, suggesting that hemo-concentration might be occurred. We also observed significant increases in lactate, CK, GH, and cortisol levels immediately after RT. Evidence has accumulated that circulating GH and insulin-like growth factor I play crucial roles in growth, development, and maintenance of skeletal muscle (Ghanbari-Niaki, Nabatchian, & Hedayati, 2007; Hoffman et al., 2008; West & Phillips, 2012), strongly suggesting that our present RT protocol is as effective for inducing GH and lactate response, and enhancing MPS or MPB.

On the other hand, there were no significant changes in urinary 3-MetHis excretion between the three groups in our study. Several methodological and physiological factors may influence the measurements, including protein (Candow et al., 2008), and carbohydrate (CHO) (Roy, Fowles, Hill, & Tarnopolsky 2000; Bird, et. al., 2006) supplementation during and/or after RT. Candow et al. (2008) examined the effect of low-dose creatine and protein supplementation during RT (3 day/wk for 10 wk), and reported that creatine and protein supplementation induced a significant decrease in 3-MetHis compared with an increase of placebo ($p < 0.05$). Additionally, Roy et al. (2000) provided iso-energetic CHO (1 g/kg BW) and CHO/protein/fat supplements and placebo immediately and 1-hour following RT, and reported that no significant differences were observed for urinary 3-MetHis between the trials. Therefore, we used a cross-over design to control for inter-individual variations in the contributions of skeletal muscle vs. non-skeletal muscle protein to urinary 3-MetHis excretion. In addition, this study was undertaken under the meat-free conditions because it is difficult to determine whether marked suppression of MPB occurs due to protein intake.

Bird et al. (2006) also reported that blood biochemistry, insulin, cortisol, and urinary 3-MetHis excretion were affected by the CHO treatment during the exercise, and CHO-induced suppression of cortisol release contributed to the reduction in 3-MetHis excretion, but not EAA. The result is possibly the same case in our present study: changes in serum cortisol level were minor, daily meals containing higher CHO could have led to attenuate an acute RT-induced increase in cortisol, and this probably concern with small changes in 3-MetHis excretion. In fact, Roy, Tarnopolsky, MacDougall, Fowles, and Yarasheski (1997) reported that a CHO supplement consumed immediately after RT (1 g/kg BW) decreases urinary 3-MetHis excretion.

In a point of supplement timing, Candow, Chilibeck, Facci, Abeysekara, and Zello (2006) determined the effects of protein supplementation immediately before (PRO-B) and after (PRO-A) RT for 12 weeks in older men, and observed that there were no significant changes in 3-MetHis. In turn, 3-MetHis excretion might not be in dependent of supplement timing.

Assessing of muscle damage can also use the perceived MS scores, and these scores elevated after an RT bout. We hypothesized that pre-RT supplementation with E or S protein would reduce skeletal muscle damage and perceived soreness: because of the net effect of RT to induce muscle hypertrophy is to shift net protein balance to a more positive value (Phillips et al., 2005), net protein balance remains negative in the absence of feeding and feeding stimulates MPS to an extent where net protein balance becomes positive, for a transient time. However, our data showed that perceived MS peaked at 24-hours post-RT with or without protein supplementation. These peak MS times are consistent with observations by Nelson, Conlee, and Parcell (2004) and Buckley et al. (2010), but not with other reports (Green, Corona, Doyle, & Ingalls, 2008; Howatson et al., 2012). There are conflicting data on MPB or MS with protein supplementation, suggesting the possibility that in contrast to our hypothesis, protein supplementation might have no effect on various MPB indices. Potential explanations for these negative findings include time (pre-RT, during, after RT) and duration of protein supplementation, with or without CHO supplementation and the type of exercise (downhill training, eccentric, ultra-marathon).

Surprisingly, UN excretion increased significantly in both protein supplementation groups compared with the C group. UN is a major vehicle for elimination of nitrogen arising from AA in the body, and UN excretion increases in response to increased dietary protein intake in humans (Young, El-Khoury, Raguso, Forslund, & Hambraeus, 2000). This suggested that pre-exercise protein consumption or dose in excess would ultimately lead to oxidative loss of the MPS response to RT.

There are several limitations of this study. First, the study participants were all untrained individuals. In addition, we had no positive control and could not properly determine under our protocol. Secondly, we did not set up a no-breakfast control group. Thirdly, we examined only the acute response following RT, and we cannot address long-term effects. Finally, our sample size was too small, which could have caused large variations in 3-MetHis. Previous studies have used nearly the same samples as our present studies (Moore et al., 2009; Howatson et al., 2012; Bird et al., 2006). Despite these limitations, our results clearly showed that the present experimental protocol properly induced muscle damage with no significant influence of E protein supplementation, and pre-exercise protein supplementation may accelerate protein catabolism.

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Conflict of Interest

The authors declare no conflict of interest.

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