

ORIGINAL

Effects of dietary soy protein on skeletal muscle volume and strength in humans with various physical activities

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Abstract : Background : In recent years, the number of bedridden people is rapidly increasing due to aging or lack of exercise in Japan. This problem is becoming more serious, since there is no countermeasure against it. In the present study, we designed to investigate whether dietary proteins, especially soy, had beneficial effects on skeletal muscle in 59 volunteers with various physical activities. **Methods :** We subjected 59 volunteers with various physical activities to meal intervention examination. Persons with low and high physical activities were divided into two dietary groups, the casein diet group and the soy diet group. They ate daily meals supplemented with 7.8 g of powdered casein or soy protein isolate every day for 30 days. Bedridden patients in hospitals were further divided into three dietary groups : the no supplementation diet group, the casein diet group and the soy diet group. They were also subjected to a blood test, a urinalysis, magnetic resonance imaging analysis and muscle strength test of the knee before and after the meal intervention study. **Results :** Thirty-day soy protein supplementation significantly increased skeletal muscle volume in participants with low physical activity, compared with 30-day casein protein supplementation. Both casein and soy protein supplementation increased the volume of quadriceps femoris muscle in bedridden patients. Consistently, soy protein significantly increased their extension power of the knee, compared with casein protein. Although casein protein increased skeletal muscle volume more than soy protein in bedridden patients, their muscle strength changes by soy protein supplementation were bigger than those by casein protein supplementation. **Conclusions :** The supplementation of soy protein would be one of the effective foods which prevent the skeletal muscle atrophy caused by immobilization or unloading. *J. Med. Invest.* 62 : 177-183, August, 2015

Keywords : bedridden patients, casein, muscle volume, quadriceps femoris muscle, soy protein isolate

INTRODUCTION

In recent years, the number of bedridden people is rapidly increasing due to aging or lack of exercise in Japan. This problem is becoming more serious. Many ubiquitin ligases, such as Muscle RING-Finger Protein-1 (MuRF-1) and Muscle atrophy F-box protein-1 (MAFbx-1)/atrogen-1, are associated with skeletal muscle atrophy (1-3). We also showed that Casitus B cell lymphoma-b (Cbl-b) ubiquitinated and degraded insulin receptor substrate-1 (IRS-1) in skeletal muscles during unloading conditions (4), suggesting that Cbl-b is one of enzymes associated with muscle atrophy. However, there is no effective drug or diet on such muscle atrophy in human.

To conquer this problem, we found that the phosphorylated pentapeptide, DGpYMP, which named Cblin (Cbl-b inhibitor), inhibited Cbl-b-mediated degradation of IRS-1, resulting in suppression of denervation-mediated muscle atrophy in mice (4). We also showed that dietary soy glycinin, which contained the Cblin-like sequence, DIYNP, effectively Cbl-b mediated IRS-1 ubiquitination in vitro (5). Dietary soy glycinin protein also prevented denervation-induced muscle atrophy in mice by inhibiting the ubiquitination

and degradation of IRS-1 (5). Based on these findings, we demonstrate that soy is an effective protein source against muscle atrophy at least in rodent animals.

In the present study, we designed to investigate whether dietary proteins, especially soy, had beneficial effects on skeletal muscle in volunteers with various physical activities. Soy protein supplementation tended to increase the volume and strength of quadriceps femoris muscle in participants with low physical activity and bedridden patients, compared with no supplementation or casein protein supplementation. Our results suggest that soy protein is a beneficial dietary source for human health under muscle proteolytic conditions, such as low physical activity and bedrest.

MATERIALS AND METHODS

Subjects with various physical activities

Fifty nine volunteers in the following groups took part in this research. First group is persons with low physical activity, who daily did desk works and hardly did sports. They were recruited from workers and students in the University of Tokushima and were not associated with this project. Second group is people with high physical activity. They were members of a citizens' marathon club and did long-distance running (> 10 km) at least twice per a week. Third group is bedridden patients in Tokushima University Hospital-associated private hospitals, such as the Inatsugi Orthopedic Hospital and Kyoritsu Hospital. They were estimated by

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performance status scoring (6) as PS 2 or 3 score : they lived on the bed or wheel chair about half period of whole day. All participants in this study did not have kidney disease, nerve or muscle disease and recent treatment with corticosteroids.

Meal intervention examination

Persons with high and low physical activities were divided into two dietary groups, the casein diet group and the soy diet group. They ate daily meals supplemented with 7.8 g of powdered casein (calorie, 30 kcal ; net component : protein, 7.5 g ; fat, 0.0 g ; carbohydrate, 0.0 g) or soy protein isolate (SPI) (calorie, 28 kcal ; net component : protein, 7.1 g ; fat, 0.1 g ; carbohydrate, 0.2 g) every day for 30 days. The powdered casein and SPI were kind gifts from Tatura Co-Operative Dairy Co. Ltd. (Morrinsville, New Zealand) and Fuji Oil Co. (Osaka, Japan), respectively.

Bedridden patients in hospitals were divided into three dietary groups : the no supplementation diet group, the casein diet group and the soy diet group. Dieticians in the hospitals served ordinary meals to bedridden patients in the no supplementation diet group. They also served the casein and soy diets, which were daily supplemented with 7.8 g of powdered casein and SPI to the lunch of ordinary meals for 30 days. Quantity of every time meal was recorded by dietician.

Health conditions of the participants were monitored by doctors or nurses in the Tokushima University Hospital, the Inatsugi Orthopedic Hospital or the Kyoritsu Hospital, during experimental period. They were also subjected to a blood test, a urinalysis, magnetic resonance imaging (MRI) analysis and muscle strength test of the knee before and after the meal intervention study, as described below.

This study was approved by the Ethics Committee of Tokushima University Hospital, and was conducted in accordance with the Declaration of Helsinki. We also obtained informed consent from all participants by explaining the purpose and risks of the study. This study was registered at the following website of the Office for Human Research Protections in Japan (www.hhs.gov/ohrp/index.html).

Assessment of daily nutrient intakes

Subjects with low and high physical activities were instructed to record the details of their diet on brief-type self-administered diet history questionnaires (BDHQ) (7). Their estimated intake of nutrients were calculated based on the answer. The intake nutrients of bedridden patients were calculated from hospital meal menu.

MRI analysis

MRI analysis was performed for the calculation of the volume of the quadriceps femoris muscle with a MRI apparatus (EXCELART Vantage 1.5T, Toshiba Medical Corp., Tokyo, Japan) in the Inatsugi Orthopedic or Kyoritsu Hospital (8). Consecutive transaxial T1-weighted images (spin-echo ; TR=400 ms, TE=15 ms, matrix size 256×352, slice thickness 5 mm ; interslice gap 5 mm) of the thighs were taken before and after the meal intervention examination. One scan of MRI included about forty images. All images were taken after approximately 15 min of rest to avoid fluid shifts that might induce interstitial or intracellular volume changes. For quantification of the quadriceps femoris muscle volume, we configured the three-dimensional imaging from the two-dimensional imaging on the MRI, using the AquariusNET software package (Terarecon Inc. Foster City, USA) (Fig. 1).

Measurement of muscle strength

To assess strength of quadriceps femoris muscle, the extension power of the knee was measured by a physical therapist with a portable muscle force dynamometer, MicroFET2 (Hoggan Health Industrial Inc. Salt Lake City, USA), as described by Bohannon

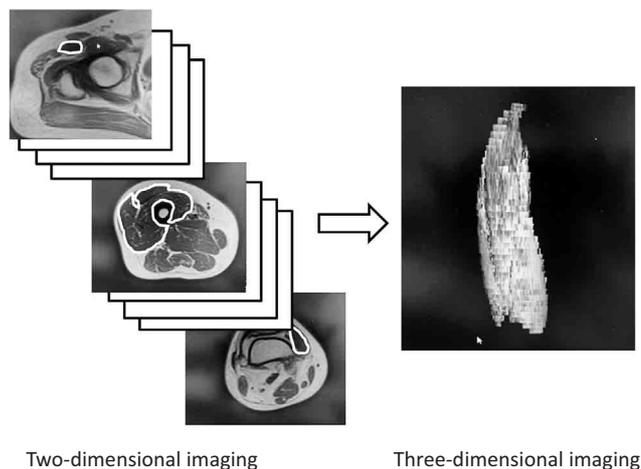


Fig. 1. Configuration of three-dimensional imaging from MRI. The subjects had a MRI of bilateral of lower extremities between hip joint and knee joint. One MRI scan included about forty images. Analysis was performed using the AquariusNET software package (Terarecon Inc. Foster City, USA). The AquariusNET software changed the traced quadriceps femoris muscle on the MRI to the three-dimensional imaging.

et al. (9). Participants were tested while they were lied upward. Their knees at the corner of the bed were at about 90 degrees of flexion (i.e., their legs were vertical). A physical therapist placed the calibrated MicroFET2 against the anterior legs of participants just proximal to the malleoli. Participants were asked to take a second or two to come to maximal effort and to then continue trying to straighten their knee as hard as possible until the tester asked them to stop (about 4 seconds later).

Biochemical blood tests

The 24-hour urine of participants were collected twice before and after the trial, and the volumes were measured. Amounts of 3-methylhistidine in the urine were measured by high performance liquid chromatography method (10). Concentrations of 8-hydroxydeoxyguanosine (8-OHdG) in the urine was also determined by on-Chip Enzyme-linked immuno-sorbent assay (11). To monitor renal functions of participants, blood urea nitrogen (BUN) and serum creatinine levels were determined as described previously (12).

Statistics

All data were statistically evaluated by ANOVA using a software Excel (Microsoft Japan, Tokyo) and its add-in software Excel Toukei Ver.7.0 (Esumi Co. Ltd. Tokyo, Japan). Differences were assessed with Scheffe's multiple comparison test and considered significant at $P < 0.05$.

RESULTS

Physical characteristics of participants with various physical activities

Physical characteristics of participants in this study was shown in Table 1. Average age in participants with various physical activities were completely different. Especially the age of participants with low physical activity was significantly lower than those of other two groups. Since this study was designed to elucidate the distinct effects of dietary proteins, it was negligible as far as there was no difference among the diet groups. There was no significant difference in height, weight and body mass index of all participants.

Table 1
Physical characteristics of participants with various physical activities.

	High physical activity				Low physical activity				Bedridden					
	Casein (n=10)		Soy (n=10)		Casein (n=7)		Soy (n=4)		No Supplementation (n=7)		Casein (n=9)		Soy (n=12)	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
%Female	70		80		0		0		57		67		50	
Age (yr)	59±2		62±2		26±2		23±0		76±5		76±6		70±6	
Height (m)	1.58±3		1.60±2		1.69±0.01		1.69±0.02		1.54±0.04		1.52±0.04		1.55±0.04	
Weight (kg)	53±3	53±2	53±4	53±2	60±3	60±3	56±4	56±4	52±4	52±4	48±4	48±4	54±5	54±5
<i>p</i>	0.98		0.9		0.92		0.94		0.95		0.97		0.97	
BMI (kg/m ²)	21±1	21±1	21±1	21±1	21±1	20±1	21±1	20±1	22±1	22±1	21±1	21±1	22±1	22±1

BMI, body mass index
Values are expressed as mean±SE.

The 30-day-intervention meal examination in this study did not change the weight and body mass index (BMI) of all participants.

Nutrient intakes of participants with various physical activities

Table 2 showed daily nutrient intakes of each group before and after the meal intervention examination, which did not contain nutrients in supplemented casein (calorie, 30 kcal/day ; protein, 7.5 g/day ; fat, 0.0 g/day ; carbohydrate, 0.0 g/day) and SPI powders (calorie, 28 kcal/day ; protein, 7.1 g/day ; fat, 0.1 g/day ; carbohydrate, 0.2 g/day). Intakes of nutrients other than nutrients in supplemented casein or SPI powder did not changed, indicating that dietary life style of participants was not affected by our meal intervention during experimental period.

Effects of dietary protein on muscle volume and muscle strength of participants with various physical activities

In participants with high physical activity, dietary soy and casein protein supplementation did not change the volume of quadriceps femoris muscle (Fig. 2A). Interestingly, 30-day soy protein supplementation significantly increased skeletal muscle volume in participants with low physical activity, compared with 30-day casein protein supplementation (Fig. 2B). In contrast, both casein and soy protein supplementation increased the volume of quadriceps femoris muscle in bedridden patients, whereas only change in skeletal muscle volume by casein supplementation was statistically significant (Fig. 2C).

Dietary soy and casein protein supplementation did not affect the extension power of the knee in participants with high physical activity (Fig. 3A). Consistent with the increased muscle volume by soy protein supplementation in participants with low physical activity, soy protein significantly increased their extension power of the knee, compared with casein protein (Fig. 3B). Although casein protein increased skeletal muscle volume more than soy protein in bedridden patients, their muscle strength changes by soy protein supplementation were bigger than those by casein protein supplementation (Fig. 3C). These changes in muscle strength were not significant, compared with that in the no supplementation group.

Effects of dietary protein on urinary 3-methylhistidine and 8-OHdG of participants with various physical activities

We measured urinary 3-methylhistidine amount per a day as a marker for muscle protein degradation (13). Before meal intervention examination, urinary 3-methylhistidine amount in bedridden patients was significantly lower than that in participants with low or high physical activity (Fig. 4A), indicating that muscle protein turnover in bedridden patients was suppressed. In participants with low physical activity, casein protein supplementation significantly increased urinary 3-methylhistidine amount, whereas soy protein supplementation did not affect the amount (Fig. 4B). In participants with high physical activity and bedridden patients, soy and casein protein supplementation did not affect urinary 3-methylhistidine amount (Fig. 4A, C)

Table 2
Nutrient intakes of participants with various physical activities.

	High physical activity				Low physical activity				Bedridden					
	Casein (n=10)		Soy (n=10)		Casein (n=7)		Soy (n=4)		No Supplementation (n=7)		Casein (n=9)		Soy (n=12)	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Calories (kcal/day)	2153±298	2048±246	1809±213	1756±170	2271±182	2190±197	2099±160	2343±295	1568±62	1568±62	1498±110	1498±110	1520±62	1520±62
<i>p</i>	0.76		0.83		0.78		0.55		1.00		1.00		1.00	
Protein (g/day)	79±11	76±8	75±8	66±5	79±7	81±8	79±4	94±11	62±3	62±3	58±5	58±5	62±3	62±3
<i>p</i>	0.79		0.32		0.87		0.3		1.00		1.00		1.00	
Fat (g/day)	61±8	56±6	53±5	48±4	68±7	72±8	69±4	94±11	43±2	43±2	41±4	41±4	42±2	42±2
<i>p</i>	0.5		0.46		0.75		0.41		1.00		1.00		1.00	

The values on this table did not include the calorie and net nutritional components of 7.8 g powdered casein (calorie, 30 kcal ; net component : protein, 7.5 g ; fat, 0.0 g ; carbohydrate, 0.0 g) or SPI (calorie, 28 kcal ; net component : protein, 7.1 g ; fat, 0.1 g ; carbohydrate, 0.2 g)
Values are expressed as mean±SE.

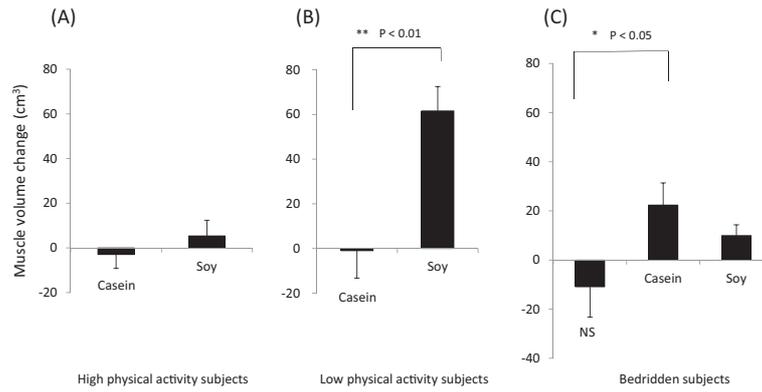


Fig. 2. Effects of dietary protein on muscle volume of participants with various physical activities

We subjected 58 volunteers with various physical activities to meal intervention examination. Persons with high (A) and low (B) physical activities were divided into two dietary groups, the casein diet group and the SPI diet group. They ate daily meals supplemented with 7.8 g of powdered casein or SPI every day for 30 days. (C) Bedridden patients in hospitals were further divided into three dietary groups: the no supplementation diet group, the casein diet group and the SPI diet group. They were also subjected to resonance imaging (MRI) analysis before and after meal intervention study. MRI analysis was performed for the calculation of the volume of the quadriceps femoris muscle with a MRI apparatus. Values are means \pm SE. NS, No Supplementation

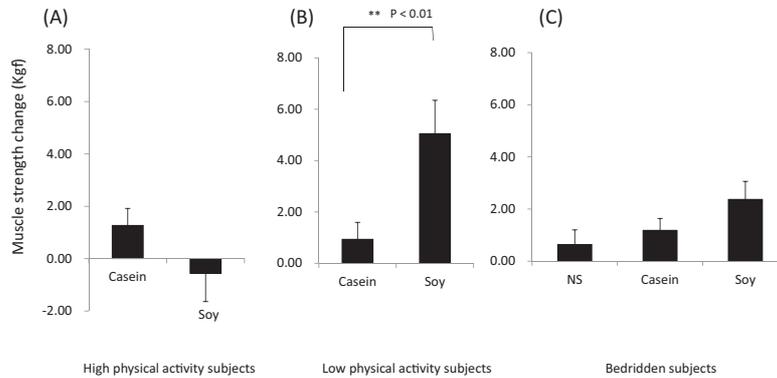


Fig. 3. Effects of dietary protein on muscle strength of participants with various physical activities

We subjected 58 volunteers with various physical activities to meal intervention examination. Persons with high (A) and low (B) physical activities were divided into two dietary groups, the casein diet group and the SPI diet group. They ate daily meals supplemented with 7.8 g of powdered casein or SPI every day for 30 days. (C) Bedridden patients in hospitals were further divided into three dietary groups: the no supplementation diet group, the casein diet group and the SPI diet group. They were also subjected to muscle strength test of the knee before and after meal intervention study. Values are means \pm SE. NS, No Supplementation

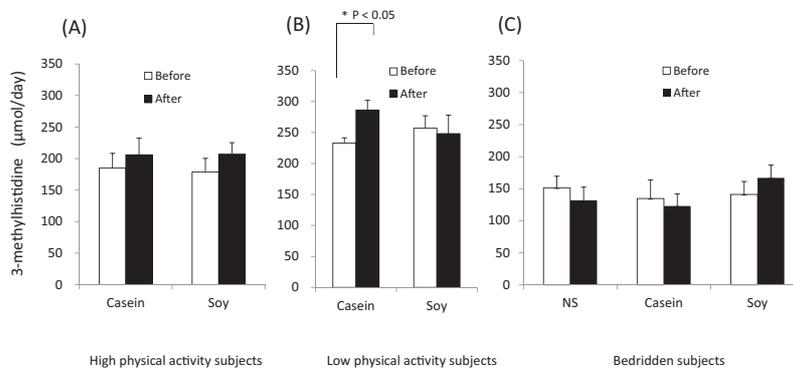


Fig. 4. Effects of dietary protein on urinary 3-methylhistidine amounts of participants with various physical activities

We subjected 58 volunteers with various physical activities to meal intervention examination. Persons with high (A) and Low (B) physical activities were divided into two dietary groups, the casein diet group and the SPI diet group. They ate daily meals supplemented with 7.8 g of powdered casein or SPI every day for 30 days. (C) Bedridden patients in hospitals were further divided into three dietary groups: the no supplementation diet group, the casein diet group and the SPI diet group. Urinary 3-methylhistidine amounts of all participants were measured before and after meal intervention study.

Values are means \pm SE. NS, No Supplementation

Urinary 8-OHdG concentration was measured to assess oxidative stress in the body (14). Interestingly, urinary 8-OHdG concentration in bedridden patients was significantly higher than that in participants with low or high physical activity, indicating that bedridden conditions induced oxidative stress in human body (Fig. 5A-C). While ordinary hospital diet tended to increase urinary 8-OHdG concentration in bedridden patients, soy or casein protein supplementation suppressed this increased 8-OHdG concentration. Urinary 8-OHdG concentration in participants with low or high physical activity was not changed by these protein supplementation.

No effect of dietary casein or soy protein on renal function of participants

BUN and serum creatinine concentrations in all participants were monitored during our experiments (Table 3), because dietary protein supplementation occasionally induces renal dysfunction (15). Any protein supplementation used in this study did not increase BUN and serum creatinine concentrations in all participants, suggesting that approximate 8 g protein supplementation per day hardly affect renal function even in elder people.

DISCUSSION

This clinical study is designed to elucidate whether soy protein supplementation has beneficial effects against skeletal muscle atrophy in human. The present results showed that muscle volume and strength in participants with low physical activity and bedridden patients were increased by soy protein supplementation. This is the first evidence, to our knowledge, that dietary soy protein had beneficial effects especially on bedridden patients with PS 2 or 3 score.

SPI contains glycinin with the Cblin-like sequence, DIYNP (5) as well as a high amount of isoflavons (16). Dietary soy glycinin prevents denervation-mediated muscle atrophy in mice through the inhibition of Cbl-b-mediated IRS-1 ubiquitination (5). Isoflavones suppress myotube atrophy in mouse skeletal muscle cells through the suppression of MuRF-1 promoter activity (16). Based on these findings, we suggest that soy contains beneficial nutrients for preventing muscle protein degradation. This finding supports the concept that soy protein is a beneficial dietary source for human health under muscle proteolytic conditions, such as low physical activity and bedrest. In contrast, casein protein supplementation had small effect on muscle volume and strength even in participants with low physical activity and bedridden patients. In this study,

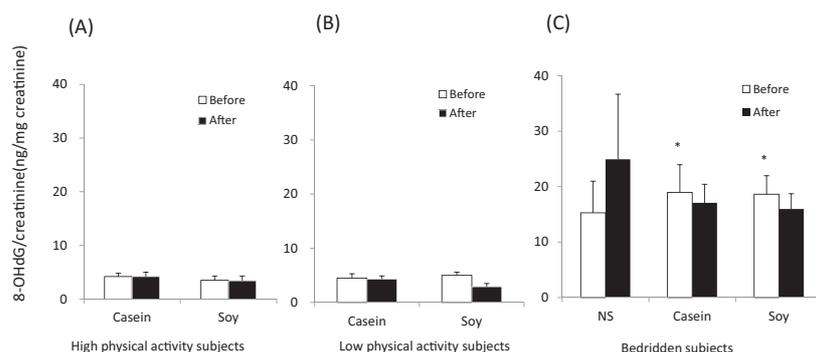


Fig. 5. Effects of dietary protein on urinary 8-OHdG concentrations of participants with various physical activities. We subjected 58 volunteers with various physical activities to meal intervention examination. Persons with high (A) and low (B) physical activities were divided into two dietary groups, the casein diet group and the SPI diet group. They ate daily meals supplemented with 7.8 g of powdered casein or SPI every day for 30 days. (C) Bedridden patients in hospitals were further divided into three dietary groups: the no supplementation diet group, the casein diet group and the SPI diet group. Urinary 8-OHdG concentrations of all participants were measured before and after meal intervention study.

Values are means ± SE. NS, No Supplementation
*indicated significant difference vs before meal intervention examination in the groups with high and low physical activities (P < 0.05)

Table 3
Blood urea nitrogen and serum creatinine levels in participants with various physical activities.

	High physical activity				Low physical activity				Bedridden					
	Casein (n=10)		Soy (n=10)		Casein (n=7)		Soy (n=4)		No Supplementation (n=7)		Casein (n=9)		Soy (n=12)	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
BUN (mg/dl)	15 ± 0.9	17 ± 1.3	18 ± 2.0	18 ± 1.9	16 ± 0.7	15 ± 1.0	15 ± 0.5	14 ± 0.8	16 ± 1.5	13 ± 0.5	15 ± 1.6	16 ± 2.1	14 ± 1.1	15 ± 1.2
<i>p</i>	0.35		0.87		0.64		0.25		0.07		0.53		0.28	
Cr (mg/dl)	0.7 ± 0.04	0.7 ± 0.04	0.7 ± 0.04	0.7 ± 0.04	0.8 ± 0.03	0.8 ± 0.02	0.8 ± 0.03	0.8 ± 0.04	0.8 ± 0.06	0.7 ± 0.06	0.7 ± 0.11	0.7 ± 0.10	0.7 ± 0.05	0.7 ± 0.05
<i>p</i>	0.55		0.81		0.74		0.3		0.55		0.96		0.67	

Values are expressed as mean ± SE.
BUN, Blood urea nitrogen ; Cr, Serum creatinine.

casein supplementation did not change muscle strength of bedridden patients, although it increased their muscle volume. Recently, Martin *et al.* (17) reported that whey proteins were more efficient than casein in the recovery of muscle functional properties following a casting-induced muscle atrophy in rats. Casein diet showed slower recovery of isometric force and concentric power, compared with whey diet. They suggested that such characteristics of casein diet were related to its less effect on muscle protein synthesis. Indeed, whey protein is known to be digested faster than casein, i.e. It has a rapid absorption kinetic, but also a higher leucine content (18, 19). Unfortunately, the current experimental settings do not allow to verify muscle protein degradation and synthesis directly, since we could not receive the consent to perform muscle biopsy.

In participants with high physical activity, any tested dietary proteins did not have any effects on muscle volume and strength. The running training at more than 10 km running/day twice a week was enough to regulate muscle protein turnover. Under such conditions, the quantity of dietary protein is presumably more important than the quality. In contrast, under the suppressed conditions of protein turnover, such as bedrest, the quality as well as the quantity of dietary protein are important to prevent the muscle atrophy. Furthermore, daily supplementation of 7.8 g protein tested in this study did not induce any renal dysfunction. Although ordinary meals of the hospitals were prepared according to the Guideline of Dietary Intakes for Japanese 2015 ([http : //www.mhlw.go.jp/bunya/kenkou/syokuji_kijyun.html](http://www.mhlw.go.jp/bunya/kenkou/syokuji_kijyun.html)), we recommend that approximately 10 g per day of dietary protein is supplemented to ordinary meals even for elder Japanese people.

Cross sectional area (CSA) on two-dimensional MRI is occasionally used to evaluate muscle volume noninvasively. However, its measurement is an inaccurate method, because the two-dimensional imaging is easily affected by the position of the subject. Therefore, for adequate quantification of the quadriceps femoris muscle volume, we configured the three-dimensional imaging from the two-dimensional imaging on the MRI, using the AquariusNET software package. The volumes of interest skeletal muscle was correctly measured using this three-dimensional imaging analysis, even if MRI was performed at different hospital. Furthermore, we found that the MRI of skeletal muscle in bedridden patients eating the casein diet was slightly bright, compared with that in bedridden patients eating SPI (unpublished data). Dietary soy protein may affect lipid metabolism in skeletal muscle, since more recently it has been reported that soy protein improved the metabolic abnormalities in the liver and skeletal muscle of dyslipemic insulin resistant rats (20). Further examinations are necessary to elucidate this hypothesis.

TEXT FOOTNOTES

1. Abbreviations

BDHQ : brief-type self-administered diet history questionnaires
 BMI : body mass index
 BUN : blood urea nitrogen
 Cbl-b : Casitus Bcell lymphoma-b
 Cblin : Cbl-b inhibitor
 CSA : Cross sectional area
 IRS-1 : Insulin receptor substrate-1
 MAFbx : Muscle atrophy F-box protein-1
 MRI : magnetic resonance imaging
 MuRF-1 : Muscle RING-Finger Protein-1
 SPI : soy protein isolate
 8-OHdG : 8-hydroxydeoxyguanosine

2. Grants

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