Amino Acids (2009) 37:609–616
DOI 10.1007/s00726-008-0180-0

ORIGINAL ARTICLE

Regulation of muscle protein degradation, not synthesis, by dietary leucine in rats fed a protein-deficient diet

Takayuki Sugawara · Yoshiaki Ito · Naoyuki Nishizawa · Takashi Nagasawa

Received: 3 July 2008 / Accepted: 29 August 2008 / Published online: 12 September 2008

Abstract The aim of this study was to elucidate the effects of long-term intake of leucine in dietary protein malnutrition on muscle protein synthesis and degradation. A reduction in muscle mass was suppressed by leucine-supplementation (1.5% leucine) in rats fed protein-free diet for 7 days. Furthermore, the rate of muscle protein degradation was decreased without an increase in muscle protein synthesis. In addition, to elucidate the mechanism involved in the suppressive effect of leucine, we measured the activities of degradation systems in muscle. Proteinase activity (calpain and proteasome) and ubiquitin ligase mRNA (Atrogin-1 and MuRF1) expression were not suppressed in animals fed a leucine-supplemented diet, whereas the autophagy marker, protein light chain 3 active form (LC3-II), expression was significantly decreased. These results suggest that the protein-free diet supplemented with leucine suppresses muscle protein degradation through inhibition of autophagy rather than protein synthesis.

Keywords Skeletal muscle · Protein synthesis · Protein degradation · Leucine · Sarcopenia · Autophagy

T. Sugawara
United Graduate School of Agricultural Sciences,
Iwate University, Morioka,
Iwate 020-8550, Japan

Y. Ito · N. Nishizawa · T. Nagasawa (✉)
Biological Chemistry and Food Science,
Faculty of Agriculture, Iwate University,
Morioka, Iwate 020-8550, Japan
e-mail: tnaga@iwate-u.ac.jp

Introduction

A characteristic of several diseases, aging and nutritional deficiency is a decrease in skeletal muscle mass resulting from a decreased rate of muscle protein synthesis and an accelerated rate of muscle protein degradation (Thomas 2007). A reduction in muscle mass results in decreased mobility and a concomitant impairment of daily activity. Therefore, it is important to maintain muscle mass by regulating muscle protein synthesis and degradation. Several hormones, as well as nutrition, are involved in regulating muscle protein metabolism (Sugden et al. 1991). Studies have shown that dietary protein and essential amino acids enhance muscle growth (Yoshizawa et al. 1999; Smith et al. 1998). Notably, branched-chain amino acids, especially leucine, are known to maintain muscle mass due to the stimulation of muscle protein synthesis and the suppression of muscle protein degradation (Buse and Reid 1975; Matthew 2005).

We have shown that myofibrillar protein degradation was suppressed by the oral administration of leucine (135 mg/100 g body weight) (Nagasawa et al. 2002). Furthermore, we have demonstrated that long-term (7-days) feeding of leucine (protein-free diet supplemented with 1.5% leucine) suppressed muscle protein degradation with a concomitant increase in muscle mass (Sugawara et al. 2007). Thus, muscle protein degradation might be a critical factor in the regulation of muscle mass. Leucine stimulates translational activity in protein synthesis via the mammalian target of rapamycin (mTOR), which stimulates phosphorylation of the eukaryotic initiation factor 4E binding protein (4E-BP1) and ribosomal protein S6 kinase 1 (S6K1) (Kimball et al. 1999). It is possible that a protein-free diet supplemented with 1.5% leucine enhanced muscle protein synthesis, although we did not measure the rate of

protein synthesis. However, even if translation factors are activated by leucine, it is possible that the rate of muscle protein synthesis is not increased, due to depletion of amino acids for material of protein synthesis. Few reports have measured the effect of leucine on the change in the rate of muscle protein synthesis. Therefore, it is necessary to measure the rate of protein synthesis and degradation.

Several proteolytic systems; such as; calpain, ubiquitin-proteasome and autophagy-lysosome, are present in skeletal muscle. Calpain is a Ca^{2+}-dependent cytosolic cysteine proteinase, which is involved in the first step of myofibrillar proteolysis (Ono et al. 2006). The autophagy-lysosome system is reportedly involved in long-lived protein degradation. Here, proteins are initially surrounded by a double-membrane vesicle, the autophagosome, which fuses with a lysosome and degraded by the lysosomal enzyme cathepsin. The microtubule-associated protein 1 light chain 3 (LC3) which is necessary to produce autophagosomes is a very specific marker of the autophagosome (Karim et al. 2007). Ubiquitin-proteasome is an ATP-dependent system. Ubiquitin-conjugated proteins are degraded by the 26S proteasome. Several studies have suggested that the E3 ubiquitin ligases Atrogin-1 and MuRF1 have central role in muscle protein degradation (Bodine et al. 2001). Our previous study has shown that long-term leucine-supplementation suppresses myofibrillar protein degradation, although the mechanism involved requires further clarification (Sugawara et al. 2007).

The aim of the present study was to elucidate the effect of long-term intake of leucine on the rate of muscle protein synthesis and degradation. In the present study, we measured the rate of myofibrillar protein degradation, as well as the rate of muscle protein synthesis. In addition, we examined the mechanism involved in the suppression of myofibrillar protein degradation as a result of leucine-supplementation. Our results indicate that leucine suppresses the rate of muscle protein degradation through the inhibition of autophagy, but does not stimulate muscle protein synthesis.

Materials and methods

Animals and experimental design

Experiment 1 Male Wistar rats (4 weeks old) were purchased from SLC (Shizuoka, Japan). They were individually housed in stainless steel wire cages and maintained at 22°C and 55% relative humidity and a 12-h light dark cycle (6:00–18:00). They were allowed free access to water and 20% casein diet (20C) according to AIN-93G (Reeves et al. 1993) for 1 week. The rats were then randomly assigned to the following three dietary groups; PF (protein-free diet), Leu (protein-free diet supplemented with 1.5% leucine, which equals to the amount of leucine in 20C), and 20C (Table 1). The rats were fed the experimental diets ad libitum for a week. During this period, the food intake of rats fed Leu was less than that of those fed 20C ad libitum in a preliminary experiment. Therefore, we used the pair-feeding method where the amount of food intake in rats fed 20C or PF was decreased as those fed Leu, because the reduced amount of food intake may affect body weight and muscle weight. On the final day, the gastrocnemius muscle was removed from the leg to measure muscle weight. The animal care protocol in this study was approved by the Iwate University Animal Research Committee under Guidelines for Animal Experiments in Iwate University.

Experiment 2 Male Wistar rats (4 weeks old) were purchased from SLC (Shizuoka, Japan). They were allowed free access to water and 20C for 1 week. The rats were then randomly assigned to the following two dietary groups, PF and Leu (Table 1). The rats were fed the experimental diets ad libitum for 1 week as Experiment 1. On the final day, the rats were anesthetized with intraperitoneal injections (1.88 mL/kg body weight [BW]) of a mixture of ketamine (43.1 mg/mL) and xylazine (2.76 mg/mL). To measure the rate of protein synthesis, 0.5 mmol/kg BW L-[$^2H_5$]-phenylalanine (Cambridge Isotope Lab, MA, USA) was injected into the tail vein. Thirty minutes post-injection, the abdomen was opened and blood collected from the inferior vena cava. The abdominal aorta was then cut to exsanguinate the rats. The extensor digitorum longus (EDL), soleus, plantaris and gastrocnemius muscles were

Table 1 Composition of experimental diets

Composition (g/kg)	PF	Leu	20C
Casein[a]	0	0	200
Leucine[b]	0	15	0
Cystine[b]	0	0	3
Sucrose[c]	100	100	100
α-Cornstarch[a]	732.5	717.5	529.5
Soy bean oil[d]	70	70	70
Mineral mixture[e]	35	35	35
Vitamin mixture[e]	10	10	10
Choline bitartrate[d]	2.5	2.5	2.5
Cellulose[a]	50	50	50

PF protein-free diet, *Leu* PF supplemented with 1.5% leucine, *20C* 20% casein diet

[a] Oriental Yeast Co., Tokyo, Japan

[b] Ajinomoto Co., Tokyo, Japan

[c] Toyo Sugar Refining Co., Tokyo, Japan

[d] Wako Pure Chemical Industries Ltd., Osaka, Japan

[e] AIN 93 composition (Reeves et al. 1993)

then removed. The collected blood was centrifuged at 3,500*g* for 15 min to obtain plasma, which was frozen in liquid nitrogen and stored at −80°C for further analysis. EDL muscle was used for measuring the rate of protein degradation, while gastrocnemius muscle was used for measuring the rate of protein synthesis, enzyme activities, Northern blot, and Western blot.

Muscle protein synthesis

The rate of muscle protein synthesis was measured using the flooding dose method (Debras et al. 2007; Rieu et al. 2007; Welle et al. 2006). Gastrocnemius muscle was homogenized with 10 volumes of 3.5% perchloric acid using a Polytron homogenizer (kinematica GmbH, Steinhofhalde, Switzerland), and the homogenates were centrifuged at 8,000*g* for 15 min. Free phenylalanine enrichment of the supernatant was assessed. The pellet was hydrolyzed with 6 M HCl at 110°C for 24 h to determine protein bound phenylalanine enrichment, and the HCl was subsequently removed by evaporation. These samples were purified using a cation exchange resin (200–400 mesh DOWEX 50 W-X8, H+ form; Bio-Rad, Richmond, CA, USA). The eluted sample was dried, and then dissolved in acetonitrile. *N*-methyl-*N*-tert-butyldimethylsilyl trifluoroacetamide (PIERCE, Rockford, IL, USA) was added to the sample and heated (80°C) for 20 min to produce the tert-butyldimethylsilyl derivative. The derivative was analyzed by GC-MS (QP2010, Shimadzu, Kyoto, Japan) (Fujita et al. 2006). Fractional synthesis rate (FSR) was calculated from the following equation.

$$\mathrm{FSR} = \mathrm{Sb} \times 100/\mathrm{Sa} \times t$$

where Sb is the enrichment at time t of the protein-bound phenylalanine, Sa is the enrichment of tissue free phenylalanine, and t is the incorporation time in a day.

Myofibrillar protein degradation

3-Methylhistidine (N^{τ}-methylhistidine, MeHis) is a sensitive index of myofibrillar protein degradation (Haverberg et al. 1975; Nagasawa et al. 1996). We incubated the isolated EDL muscle in a Krebs–Ringer bicarbonate buffer containing 10 mM glucose under 95% O_2–5% CO_2 at 37°C for 2 h after a 30 min pre-incubation at 37°C to measure the rate of MeHis release (Nagasawa et al. 1998). The amount of MeHis in the incubation buffer was measured by HPLC after the derivatization by fluorescamine and treatment with perchloric acid and heating (Wassner et al. 1980). Several studies have shown that the release of MeHis from isolated muscle for incubation reflects an individual nutritional condition (Nagasawa et al. 1996, 2002, 2004).

Concentration of plasma branched-chain amino acids (BCAA)

Plasma leucine, isoleucine, and valine concentrations were measured by HPLC after derivatization of *o*-phthalaldehyde by our previous method with a minor modification (Nagasawa et al. 1991; Sugawara et al. 2007).

Plasma insulin concentration

Plasma insulin concentration was measured using enzyme-linked immunosorbent assay (ELISA) (Morinaga Institute of Biological Science, Yokohama, Japan).

Proteinase activities in muscle

We measured proteinase activity in gastrocnemius muscle. Calpain activity was measured by the method of Higuchi et al. (1996), using azocasein as the substrate. Gastrocnemius muscle (100 mg) was homogenized with 30 volumes buffer [100 mM Tris, 1 mM EDTA (pH 7.5)] using a Polytron homogenizer, with the resultant sample used as crude enzyme.

Proteasome activity was measured by the method of Aki et al. (1994), using succinyl-Leu-Leu-Val-Tyr-MCA (chymotrypsin-like activity) as the substrate. Gastrocnemius muscle (100 mg) was homogenized with 30 volumes buffer [10 mM Tris, 1 mM EDTA, 5 mM $MgSO_4$, 250 mM Sucrose, 0.1% Brij35 (pH 7.4)] using a Polytron homogenizer, with the resultant sample used as crude enzyme.

Northern blotting for the detection of Atrogin-1 and MuRF1 expression

For the detection of mRNA transcripts by Northern blotting, specific cDNA probes were synthesized using the following oligonucleotides: rat Atrogin-1, 5′-ATCCCTGAGTGGCA TCGC-3′ and 5′-CTCTTCCACAGTAGCCGGT-3′; rat MuRF1, 5′-GGACGGAAATGCTATGGAGAT-3′ and 5′-A ACGACCTCCAGACATGGA-3′; rat GAPDH, 5′-ACCA CAGTCCATGCCATCAC-3′ and 5′-TCCACCACCCTGT TGCTGTA-3′. Total RNA was prepared by the AGPC method (Chomczynski and Sacchi 1987). Total RNA (10 μg) from rat gastrocnemius muscle was subjected to Northern blot analysis using the DIG system (Roche Diagnostics, Tokyo, Japan) as previously described (Ito et al. 2006).

Western blotting for expression of LC3

Gastrocnemius muscle (200 mg) was homogenized with 10 volumes 0.25 M sucrose/1 mM EDTA (pH 7.4) using a

Polytron homogenizer and centrifuged at 3,800*g*. The precipitate was used as the SDS-PAGE sample. The sample was separated on 15% polyacrylamide gel and transferred to a PVDF membrane (Amersham Biosciences, Tokyo, Japan). The membrane was blocked for 1 h with 5% skim milk in Tris buffered saline (TBS) buffer containing 0.1% Tween 20 (TBS-T) at room temperature. The membrane was incubated overnight at 4°C with anti-LC3B polyclonal antibodies (Cell Signaling Technology, Danvers, MA, USA) in TBS-T (containing 5% bovine serum albumin), followed by 1 h incubation with HRP-conjugated goat anti-rabbit IgG (Stressgen, Victoria, BC, Canada) in TBS-T (containing 5% skim milk). Detection of the secondary antibody was performed using an ECL western blot detection kit (Amersham Biosciences) (Karim et al. 2007). To estimate the relative intensity of each band, X-ray films were scanned and analyzed using image analysis software (NIH Image, version 1.62).

Statistical analyses

Data are expressed as means with SEM. Comparisons between animals fed a PF diet and those fed other diets were carried out using a two-tailed unpaired *t*-test (Instat Ver. 2.03). Differences were considered significant at $P < 0.05$.

Results

Experiment 1 The final body weights of rats fed Leu did not differ compared with those fed PF, but 20C were significantly heavier than those fed PF. The weight of gastrocnemius muscle (weight per body weight) were significantly increased in animals fed Leu or 20C compared with those fed PF (Table 2), indicating that long-term feeding of protein-free diet decrease muscle mass, and leucine suppresses the reduction.

Experiment 2 The final body weights of rats fed PF (84.3 ± 1.8 g) and Leu (82.3 ± 1.4 g) did not significantly differ. The total food intake of rats fed PF was 48.5 g and was slightly decreased for those fed Leu (45.7 ± 1.4 g). The muscle (EDL, soleus, plantaris and gastrocnemius) weights per body weights were significantly increased in animals fed Leu (Fig. 1). There were no significant differences between plasma branched-chain amino acids concentrations (BCAA) (valine, isoleucine, and leucine) or plasma insulin concentrations of rats fed PF or Leu (Figs. 2, 3).

Table 2 Body weight and gastrocnemius muscle weight of rats (Experiment 1)

	Body weight (g)	Muscle weight	
		(g)	(mg/g body weight)
PF	69.7 ± 1.7	0.29 ± 0.01	4.12 ± 0.11
Leu	70.8 ± 1.1	0.34 ± 0.02	4.74 ± 0.24*
20C	78.4 ± 2.3*	0.36 ± 0.02*	4.61 ± 0.10*

Value is the mean ± SE ($n = 5$). * $P < 0.05$ vs. PF

The rate of MeHis release from the isolated EDL muscle in rats fed Leu was significantly suppressed (28%) as compared with that in rats fed PF (Fig. 4a). In our previous study (Sugawara et al. 2007), the rate of myofibrillar protein degradation was increased in animals fed PF diet as compared with those fed a control diet (20% casein). Leucine suppressed an increased degradation of myofibrillar protein caused by a PF diet. On the other hand, leucine-supplementation did not change the FSR of gastrocnemius muscle (Fig. 4b). Thus, leucine-supplementation did not enhance the rate of muscle protein synthesis.

Next, we investigated how the proteolytic system is regulated by leucine. Calpain activity and chymotrypsin-like activity (proteasome) were measured in gastrocnemius muscle and were found to be unaltered in animals fed a leucine supplemented diet (Fig. 5). Because the ubiquitin-proteasome system cannot be evaluated from proteasome activity alone, we therefore measured ubiquitin ligase mRNA expression. Atrogin-1 mRNA expression was significantly increased by the feeding of Leu. MuRF1 mRNA expression showed an increasing trend in animals fed Leu ($P = 0.14$). Thus, ubiquitin ligase mRNA was not decreased but rather increased by the feeding of Leu (Fig. 6).

The change in LC3 expression, a marker of autophagosome, was evaluated by LC3-II form using Western blotting. LC3-I is the precursor form, whereas LC3-II is the active form. LC3-II expression was decreased significantly in animals fed Leu (Fig. 7). These results suggest that leucine-supplementation did not suppress the calpain and ubiquitin-proteasome proteolytic systems, but significantly suppressed the autophagy-lysosome system.

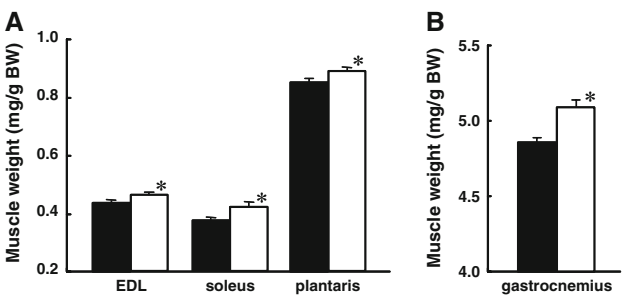

Fig. 1 Effect of feeding a protein-free diet (PF, *filled bars*) or PF diet supplemented with 1.5% leucine (Leu, *open bars*) on weight of EDL, soleus, and plantaris muscle (**a**) and gastrocnemius muscle (**b**). Values are represented as the mean ± SEM ($n = 5$). *$P < 0.05$ versus PF

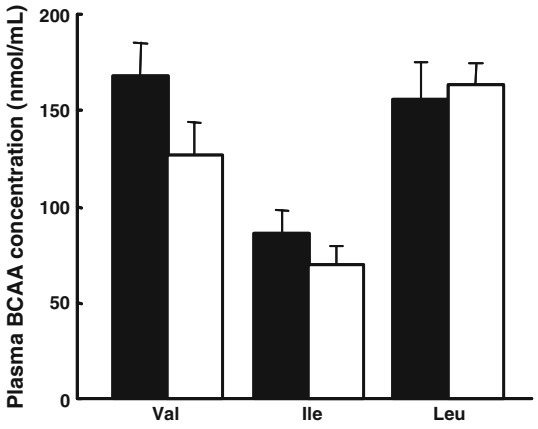

Fig. 2 Effect of feeding a protein-free diet (PF, *filled bars*) or PF diet supplemented with 1.5% leucine (Leu, *open bars*) on branched-chain amino acid concentrations in plasma. Values are represented as the mean ± SEM ($n = 5$)

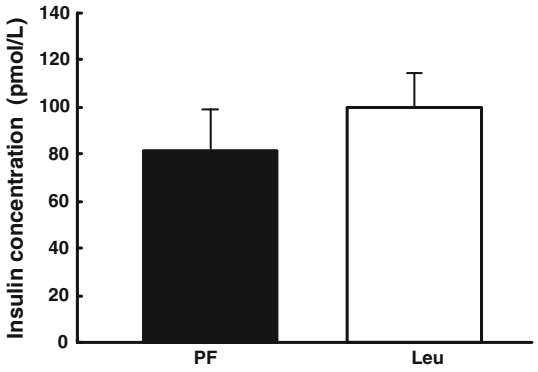

Fig. 3 Effect of feeding a protein-free diet (PF) or PF diet supplemented with 1.5% leucine (Leu) on insulin concentration in plasma. Values are represented as the mean ± SEM ($n = 5$)

Discussion

This study demonstrated that a decrease in muscle mass in animals fed a protein-free diet was suppressed by the feeding of supplemental leucine for 7 days. Furthermore, we were able to show that myofibrillar protein degradation was prominently suppressed and muscle protein synthesis was not enhanced. These results strongly suggest that muscle mass reduction is suppressed by leucine in rats fed a protein-deficient diet and is regulated by protein degradation rather than protein synthesis.

Generally, muscle mass is regulated by muscle protein synthesis and degradation. Therefore, it is important to maintain or increase muscle mass by enhancing synthesis and suppressing degradation. Many studies show that muscle protein metabolism could be regulated by nutrients. Rieu et al. (2003) showed that the feeding of a diet supplemented with leucine for 10 days stimulated muscle protein synthesis in rats. Kimball and Jefferson (2004)

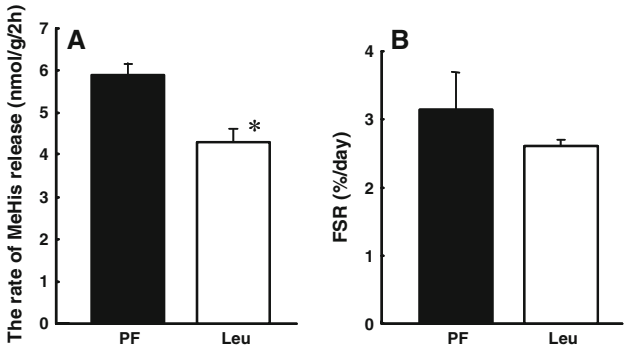

Fig. 4 Effect of feeding a protein-free diet (PF) or PF diet supplemented with 1.5% leucine (Leu) on the rate of myofibrillar protein degradation (**a**) and fractional synthesis rate (FSR) (**b**). Values are represented as the mean ± SEM ($n = 5$). *$P < 0.05$ versus PF

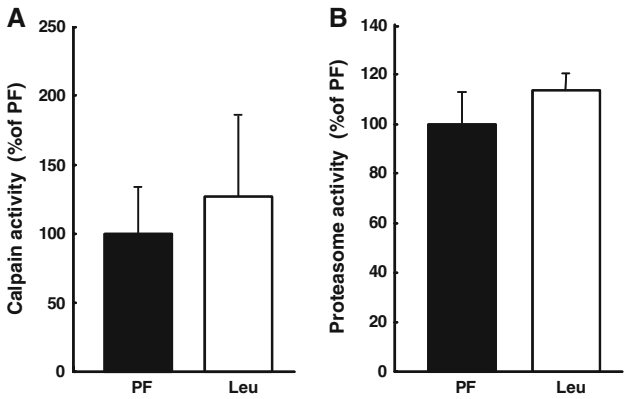

Fig. 5 Effect of feeding a protein-free diet (PF) or PF diet supplemented with 1.5% leucine (Leu) on calpain activity (**a**) and proteasome activity (chymotrypsin-like activity) (**b**). Values are represented as the mean ± SEM ($n = 5$)

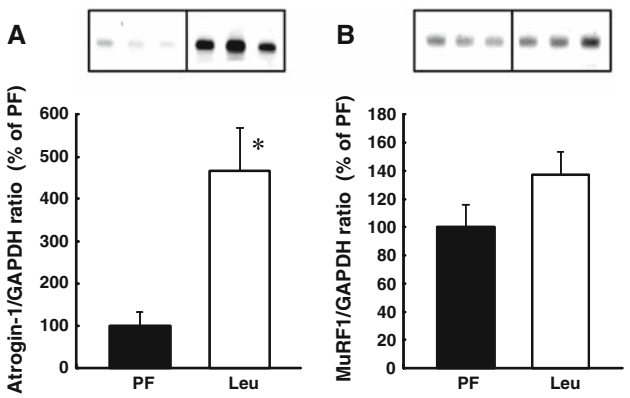

Fig. 6 Effect of feeding a protein-free diet (PF) or PF diet supplemented with 1.5% leucine (Leu) on Atrogin-1 mRNA expression (**a**) and MuRF1 mRNA expression (**b**). The data was normalized to the level of GAPDH mRNA. Values are represented as the mean ± SEM ($n = 5$). *$P < 0.05$ versus PF

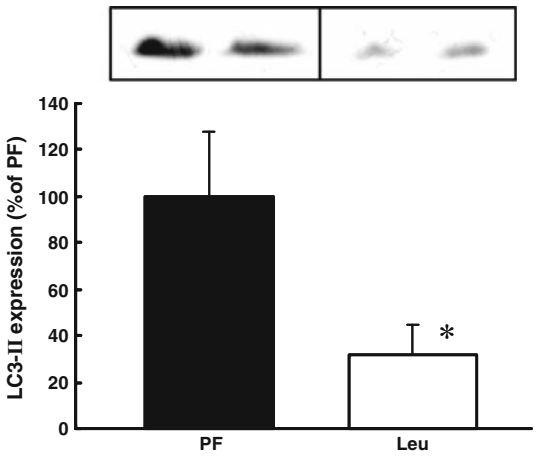

Fig. 7 Effect of feeding a protein-free diet (PF) or PF diet supplemented with 1.5% leucine (Leu) on LC3-II expression. Values are represented as the mean ± SEM ($n = 4$–5). *$P < 0.05$ versus PF

showed that muscle protein synthesis in rats was stimulated by oral administration of leucine. In these studies, the rats were fed sufficient dietary protein, and the dietary protein could be used as material to make muscle protein. Leucine stimulates protein synthesis through mTOR, thereby stimulating phosphorylation of 4E-BP1 and S6K1, which is involved in protein translation (Anthony et al. 2002). In the present study, although we did not measure the changes in these factors, it is possible that these factors were stimulated by feeding of a leucine-supplemented diet. However, the rate of muscle protein synthesis was not stimulated in animals fed a leucine-supplemented diet. This suggests that when dietary protein is deficient, the amino acids used as material to synthesis muscle protein are deficient. Although amino acids are supplied by muscle protein degradation, these might be not sufficient for protein synthesis. On the other hand, in this study, muscle protein degradation was suppressed by leucine-supplementation. Unlike an increase in the rate of protein synthesis, material is not necessary for the suppression of protein degradation. Under the present experimental conditions, muscle protein degradation is a key factor in muscle mass regulation.

Insulin is thought to enhance muscle protein synthesis and suppress degradation, and leucine is known to stimulate secretion of insulin (Li et al. 2003). In this study, however, plasma insulin concentration was not increased by leucine-supplementation. An increase in plasma insulin concentration was likely, but may have only occurred during a very short period. Therefore, suppression of muscle mass reduction and myofibrillar protein degradation seems to be independent of the action of insulin. However, other hormones such as glucocorticoid and IGF-I may affect muscle protein synthesis and degradation.

Myofibrillar proteins are degraded by the calpain, ubiquitin-proteasome and autophagy-lysosome systems. Studies have shown that the ubiquitin-proteasome system is stimulated by several catabolic conditions (Fang et al. 2000; Rajan and Mitch 2008). Several reports suggest that leucine suppresses accelerated muscle protein degradation by the inhibition of the ubiquitin-proteasome system. Nakashima et al. (2005) showed that ubiquitin and 20S proteasome C2 subunit mRNA expressions decreased in chicks orally administered leucine. Hamel et al. (2003) demonstrated that several amino acids, including leucine, directly inhibited the activity of muscular proteasome. Therefore, it is possible that suppression of muscle protein degradation by leucine may be mediated by the ubiquitin-proteasome system. In the present study, however, proteasome (chymotrypsin-like) activity which is one of index in proteasomal proteolysis and ubiquitin ligase (MuRF1 and Atrogin-1) mRNA expression were not inhibited by feeding of leucine-supplementation. Unexpectedly, Atrogin-1 mRNA expression increased significantly in animals fed Leu, but the reason has yet to be elucidated. Leucine is also known to affect the autophagy-lysosome system. Mordier et al. (2000) showed that leucine restriction induces the formation of autophagy and activation of lysosomal-dependent proteolysis in C2C12 myotube. Kadowaki and Kanazawa (2003) suggest that leucine has a direct regulatory potential in liver autophagic proteolysis. We showed in the present experiment that autophagy marker protein active form, LC3-II expression was decreased significantly in rats fed a Leu diet. These results indicate that the autophagy-lysosome system may be regulated by leucine.

We have previously shown that oral administration of leucine (135 mg/100 g body weight) increased plasma leucine concentration (2,000 nmol/ml) within 1 h, and subsequently decreased to post-absorption levels (Nagasawa et al. 2002). The rapid reduction of leucine implies that leucine is quickly oxidized (Harper et al. 1984). In the present experiment, the plasma leucine concentration was approximately 150 nmol/ml in rats fed a leucine-supplemented diet, and did not significantly differ from levels in animals fed a PF diet. It is conceivable that the plasma leucine concentration was increased immediately after feeding of a leucine-supplemented diet and returned to post-absorption levels prior to sampling. Therefore, it is possible that protein synthesis was not stimulated in the time of measurement; however, the increase in the rate of synthesis might be not occurred because of less material amino acids for synthesis.

In conclusion, we showed that feeding of a leucine-supplemented diet suppresses muscle mass reduction caused by protein deficiency. Long-term feeding of leucine suppressed myofibrillar protein degradation and did not stimulate muscle protein synthesis. Therefore, under the condition of dietary protein deficiency, regulation of

muscle protein degradation is a key factor in the suppression of muscle mass reduction. Furthermore, LC3-II expression clearly decreased. These results suggest that inhibition of autophagy-lysosome is likely the system involved in the suppression of myofibrillar protein degradation by leucine.

Acknowledgments We thank Prof. Kadowaki (Niigata University) for discussion about autophagy. This work was supported in part by Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science; the 21st century COE in Iwate University program from the Ministry of Education, Culture, Sports, Science and Technology of Japan; and Ajinomoto Co. Ltd.

References

Aki M, Shimbara N, Takashina M, Akiyama K, Kagawa S, Tamura T, Tanahashi N, Yoshimura T, Tanaka K, Ichihara A (1994) Interferon-gamma induces different subunit organizations and functional diversity of proteasomes. J Biochem (Tokyo) 115:257–269

Anthony JC, Reiter AK, Anthony TG, Crozier SJ, Lang CH, MacLean DA, Kimball SR, Jefferson LS (2002) Orally administered leucine enhances protein synthesis in skeletal muscle of diabetic rats in the absence of increases in 4E-BP1 or S6K1 phosphorylation. Diabetes 51:928–936

Bodine SC, Latres E, Baumhueter S, Lai VK, Nunez L, Clarke BA, Poueymirou WT, Panaro FJ, Na E, Dharmarajan K, Pan ZQ, Valenzuela DM, DeChiara TM, Stitt TN, Yancopoulos GD, Glass DJ (2001) Identification of ubiquitin ligases required for skeletal muscle atrophy. Science 294:1704–1708

Buse MG, Reid SS (1975) Leucine. A possible regulator of protein turnover in muscle. J Clin Invest 56:1250–1261

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenolchloroform extraction. Anal Biochem 162:156–159

Debras E, Prod'homme M, Rieu I, Balage M, Dardevet D, Grizard J (2007) Postprandial leucine deficiency failed to alter muscle protein synthesis in growing and adult rats. Nutrition 23:267–276

Fang CH, Li BG, Fischer DR, Wang JJ, Runnels HA, Monaco JJ, Hasselgren PO (2000) Burn injury upregulates the activity and gene expression of the 20 S proteasome in rat skeletal muscle. Clin Sci (Lond) 99:181–187

Fujita T, Kajita M, Sano H (2006) Responses of whole body protein synthesis, nitrogen retention and glucose kinetics to supplemental starch in goats. Comp Biochem Physiol B Biochem Mol Biol 144:180–187

Hamel FG, Upward JL, Siford GL, Duckworth WC (2003) Inhibition of proteasome activity by selected amino acids. Metabolism 52:810–814

Harper AE, Miller RH, Block KP (1984) Branched-chain amino acid metabolism. Annu Rev Nutr 4:409–454

Haverberg LN, Deckelbaum L, Bilmazes C, Munro HN, Young VR (1975) Myofibrillar protein turnover and urinary N^{τ}-methylhistidine output. Response to dietary supply of protein and energy. Biochem J 152:503–510

Higuchi K, Hayashi K, Ohtsuka A, Tomita Y (1996) Calcitonin decreases corticosterone-induced skeletal muscle calpain activity. J Nutr Sci Vitaminol (Tokyo) 42:491–496

Ito Y, Oumi S, Nagasawa T, Nishizawa N (2006) Oxidative stress induces phosphoenolpyruvate carboxykinase expression in H4IIE cells. Biosci Biotechnol Biochem 70:2191–2198

Kadowaki M, Kanazawa T (2003) Amino acids as regulators of proteolysis. J Nutr 133:2052S–2056S

Karim MR, Kanazawa T, Daigaku Y, Fujimura S, Miotto G, Kadowaki M (2007) Cytosolic LC3 ratio as a sensitive index of macroautophagy in isolated rat hepatocytes and H4-II-E cells. Autophagy 3:553–560

Kimball SR, Jefferson LS (2004) Regulation of global and specific mRNA translation by oral administration of branched-chain amino acids. Biochem Biophys Res Commun 313:423–427

Kimball SR, Shantz LM, Horetsky RL, Jefferson LS (1999) Leucine regulates translation of specific mRNAs in L6 myoblasts through mTOR-mediated changes in availability of eIF4E and phosphorylation of ribosomal protein S6. J Biol Chem 274:11647–11652

Li C, Najafi H, Daikhin Y, Nissim IB, Collins HW, Yudkoff M, Matschinsky FM, Stanley CA (2003) Regulation of leucine-stimulated insulin secretion and glutamine metabolism in isolated rat islets. J Biol Chem 278:2853–2858

Matthews DE (2005) Observations of branched-chain amino acid administration in humans. J Nutr 135:1580S–1584S

Mordier S, Deval C, Bechet D, Tassa A, Ferrara M (2000) Leucine limitation induces autophagy and activation of lysosome-dependent proteolysis in C2C12 myotubes through a mammalian target of rapamycin-independent signaling pathway. J Biol Chem 275:29900–29906

Nagasawa T, Hirano J, Yoshizawa F, Nishizawa N (1998) Myofibrillar protein catabolism is rapidly suppressed following protein feeding. Biosci Biotechnol Biochem 62:1932–1937

Nagasawa T, Kido T, Yoshizawa F, Ito Y, Nishizawa N (2002) Rapid suppression of protein degradation in skeletal muscle after oral feeding of leucine in rats. J Nutr Biochem 13:121–127

Nagasawa T, Kikuchi N, Ito Y, Yoshizawa F, Nishizawa N (2004) Suppression of myofibrillar protein degradation after refeeding in young and adult mice. J Nutr Sci Vitaminol (Tokyo) 50:227–230

Nagasawa T, Sakai T, Onodera R (1991) Simple and sensitive determination of plasma N^{τ}-methylhistidine by high-performance liquid chromatography using pre-column derivative formation with *o*-phthalaldehyde-2-mercaptoethanol. J Chromatogr 566:223–227

Nagasawa T, Yoshizawa F, Nishizawa N (1996) Plasma N^{τ}-methylhistidine concentration is a sensitive index of myofibrillar protein degradation during starvation in rats. Biosci Biotechnol Biochem 60:501–502

Nakashima K, Ishida A, Yamazaki M, Abe H (2005) Leucine suppresses myofibrillar proteolysis by down-regulating ubiquitin-proteasome pathway in chick skeletal muscles. Biochem Biophys Res Commun 336:660–666

Ono Y, Torii F, Ojima K, Doi N, Yoshioka K, Kawabata Y, Labeit D, Labeit S, Suzuki K, Abe K, Maeda T, Sorimachi H (2006) Suppressed disassembly of autolyzing p94/CAPN3 by N2A connectin/titin in a genetic reporter system. J Biol Chem 281:18519–18531

Rajan VR, Mitch WE (2008) Muscle wasting in chronic kidney disease: the role of the ubiquitin proteasome system and its clinical impact. Pediatr Nephrol 23:527–535

Reeves PG, Nielsen FH, Fahey GC Jr (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 123:1939–1951

Rieu I, Sornet C, Bayle G, Prugnaud J, Pouyet C, Balage M, Papet I, Grizard J, Dardevet D (2003) Leucine-supplemented meal feeding for ten days beneficially affects postprandial muscle protein synthesis in old rats. J Nutr 133:1198–1205

Rieu I, Balage M, Sornet C, Debras E, Ripes S, Rochon-Bonhomme C, Pouyet C, Grizard J, Dardevet D (2007) Increased availability of leucine with leucine-rich whey proteins improves postprandial muscle protein synthesis in aging rats. Nutrition 23:323–331

Smith K, Reynolds N, Downie S, Patel A, Rennie MJ (1998) Effects of flooding amino acids on incorporation of labeled amino acids into human muscle protein. Am J Physiol 275:E73–E78

Sugawara T, Ito Y, Nishizawa N, Nagasawa T (2007) Supplementation with dietary leucine to a protein-deficient diet suppresses myofibrillar protein degradation in rats. J Nutr Sci Vitaminol (Tokyo) 53:552–555

Sugden PH, Fuller SJ (1991) Regulation of protein turnover in skeletal and cardiac muscle. Biochem J 273:21–37

Thomas DR (2007) Loss of skeletal muscle mass in aging: examining the relationship of starvation, sarcopenia and cachexia. Clin Nutr 26:389–399

Wassner SJ, Schlitzer JL, Li JB (1980) A rapid, sensitive method for the determination of 3-methylhistidine levels in urine and plasma using high-pressure liquid chromatography. Anal Biochem 104:284–289

Welle S, Bhatt K, Pinkert CA (2006) Myofibrillar protein synthesis in myostatin-deficient mice. Am J Physiol Endocrinol Metab 290:E409–E415

Yoshizawa F, Kido T, Nagasawa T (1999) Stimulative effect of dietary protein on the phosphorylation of p70 S6 kinase in the skeletal muscle and liver of food-deprived rats. Biosci Biotechnol Biochem 63:1803–1805