Enhancement of Myofibrillar Proteolysis Following Infusion of Amino Acid Mixture Correlates Positively with Elevation of Core Body Temperature in Rats

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(Received April 30, 2008)

Summary  Administration of an amino acid (AA) mixture stimulates muscle protein synthesis and elevates core body temperature (Tb), as characteristically found under anesthetic conditions. We tested the hypothesis that not only AA given, but also AA produced by degradation of endogenous muscular protein are provided for muscle protein synthesis, which is further reflected in Tb modifications. Rats were intravenously administered an AA mixture or saline in combination with the anesthetic propofol or lipid emulsion. We measured plasma 3-methylhistidine (MeHis) concentrations as an index of myofibrillar protein degradation, rectal temperature and mRNA expression of atrogin-1, MuRF-1 and ubiquitin in gastrocnemius and soleus muscles of rats following 3 h infusion of test solutions. Tb did not differ significantly between conscious groups, but was higher in the AA group than in the saline group among anesthetized rats. Plasma MeHis concentrations were higher in the AA group than in the saline group under both conditions. Plasma MeHis levels correlated positively with Tb of rats under both conditions. AA administration decreased mRNA levels of atrogin-1 and ubiquitin in gastrocnemius muscle and all mRNA levels in soleus muscle. These results suggest that AA administration enhances myofibrillar protein degradation and that the change is a determinant of Tb modification by AA administration. However, the mechanisms underlying AA administration-associated enhancement of myofibrillar proteolysis remains yet to be determined.

Key Words  methylhistidine, hypothermia, anesthesia, muscle, protein metabolism

Amino acid (AA) administration or protein ingestion elevates blood temperature in healthy human subjects (1, 2). The conversion into a positive heat balance by AA administration is clinically applied to the intraoperative management of core body temperature (Tb) (3–6). Hypothermia is common during surgery due to impairment of thermoregulatory responses by anesthetic administration (7), representing one of the major causes of postoperative complications (8, 9). Given that AA administration is utilized for intraoperative thermal management, understanding the precise mechanisms by which AA induces heat accumulation in the body is crucial.

Energy expenditure is increased after AA administration compared to glucose or lipid administration, as energy derived from ingested AAs or stored nutrients is more utilized with either AA incorporation into protein or direct oxidation (10). Skeletal muscle makes a major contribution to total body protein turnover. We have previously reported that AA administration stimulates muscle protein synthesis in both conscious and anesthetized rats (11). In general, not only AA administered, but also AA liberated by endogenous protein breakdown would be utilized in part for protein synthesis. Muscle protein breakdown along with muscular protein synthesis may also affect Tb modification after AA administration. However, no studies have described whether administration of AA alone affects muscle protein breakdown under anesthetic and conscious conditions and thus whether Tb is modified.

The 3-methylhistidine (MeHis) generated by biological degradation of muscular actin and myosin is not reutilized as a material for muscular protein and is not reabsorbed by the renal tubule. Levels of urinary MeHis excretion or plasma MeHis are thus used as indices of myofibrillar protein breakdown. Plasma MeHis concentrations can reflect acute changes in the degradation of myofibrillar protein (12, 13). Measurement of plasma MeHis concentrations would thus be appropriate to elucidate relationships between degradation of myofibrillar protein and comparable early stages of nutritional changes after AA administration.

Skeletal muscle contains multiple proteolytic systems (e.g., lysosomal and non-lysosomal Ca2+- and ATP-ubiquitin-dependent pathways), each of which could be involved in metabolic protein turnover. The ubiquitin proteolytic pathway appears primarily responsible for the increased degradation of contractile components,
such as myofibrillar proteins, in skeletal muscle under various catabolic conditions (14). The ubiquitin-proteosome system first requires the targeting of specific protein substrates for degradation, which is fulfilled by the activity of a hierarchical cascade containing E1 (ubiquitin-activating), E2 (ubiquitin-conjugating) and E3 (ubiquitin-ligating) enzymes (15). The gene expression of the two muscle-specific E3 ligases: muscle RING finger (MuRF1) and muscle atrophy F-box (MAFbx; also known as atrogin-1) is extremely well studied from its relevance to muscular protein degradation following nutrient ingestion (16–18), but the precise mechanisms and physiological roles have remained to be determined. Furthermore, the contributions of the proteolytic pathway for situations in which AA mixture alone is provided have yet to be addressed.

The aim of the present study was to elucidate whether AA administration enhances myofibrillar protein degradation in anesthetized and conscious rats and whether such degradation is associated with Tn modification. Furthermore, to elucidate the mechanism underlying changes in myofibrillar protein degradation by AA administration, we also examined mRNA expression profiles of components of the ubiquitin-proteosome system in rat skeletal muscles.

**MATERIALS AND METHODS**

*Animals and surgery.* Male Sprague-Dawley rats from Charles River Japan (Yokohama, Japan) weighing 250–310 g were maintained under conditions of constant humidity and temperature (22 ± 2°C) on a 12:12 h light-dark cycle. Rats were provided with ad libitum access to standard diet and water. The following surgical and experimental procedures were approved by the Committee on the Care and Use of Laboratory Animals at Otsuka Pharmaceutical Factory, Inc. (Tokushima, Japan).

*Infusion protocol.* Infusion of the test solutions and anesthetic was conducted as described previously (11). The day before the experiment, a silicon catheter was inserted into the jugular vein and threaded proximally ~3.5 cm until a stable temperature reading was obtained after sodium pentobarbital (50 mg/kg) was administered via bolus injection through a catheter. Blood was collected from the abdominal artery with a heparinized syringe, after which the soleus and gastrocnemius muscles were rapidly excised. The blood was centrifuged at 1,800 × g for 20 min at 4°C to obtain plasma and tissues were immediately frozen in liquid nitrogen and stored at −80°C.

*Measurement of plasma creatinine and MeHis.* Plasma creatinine concentrations were measured using the creatinase-HMMPS method (L-Type Creatinine M; Wako Pure Chemical Industries, Ltd., Osaka, Japan). For determination of plasma concentrations of MeHis, the plasma sample was mixed with ethanol and then hydrolyzed with 6 N HCl at 110°C for 2 h, then concentrations of MeHis were measured by high-performance liquid chromatography (2690 Alliance separation module, Waters, Milford, MA) with a CD-C18 column (2.0×150 mm, 3 μm; Intakt Co., Kyoto, Japan) using a mobile phase of 50 mM SDS/acetonitrile/phosphate at 610 : 390 : 3 at a flow rate of 0.2 mL/min. An ultraviolet detector (2487, Waters) was used for detection at

| Amino acids | g/dL |
|-------------|------|
| L-Leucine   | 1.40 |
| L-Isoleucine| 0.80 |
| L-Valine    | 0.80 |
| L-Lysine    | 1.05 |
| L-Threonine | 0.57 |
| L-Triptophan| 0.20 |
| L-Methionine| 0.39 |
| L-Phenylalanine| 0.70 |
| L-Cystine   | 0.10 |
| L-Tyrosine  | 0.05 |
| L-Arginine  | 1.05 |
| L-Histidine | 0.50 |
| L-Alanine   | 0.80 |
| L-Proline   | 0.50 |
| L-Serine    | 0.30 |
| Glycine     | 0.59 |
| L-Aspartic acid | 0.10 |
| L-Glutamic acid | 0.10 |

Total concentration 10.00
concentrations of MeHis were determined by comparing peak height of samples with those of external standards.

**RNA isolation and real-time PCR.** Total RNA was extracted from the skeletal muscles using a taqman Rneasy® Mini kit (QIAGEN, Hilden, Germany). Total RNA samples obtained from rat tissues were diluted to 10 μg/mL with RNase-free water containing 50 μg/mL of yeast tRNA (Life Technologies, Rockville, MD, USA). Total RNA at 30 ng per 20 μl of reaction mixture was used for measurement of the target mRNA in each tissue. RT-PCR assays were performed using an Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) under the same conditions as in previous studies (19). Real-time PCR primers were designed for rat ubiquitin, atrogin-1, MuRF1 and β-actin, and the sequences of the forward primers and reverse primers were as follows:
rat ubiquitin, 5′-TGACCAGCAGAGGCTCATCTT-3′—5′-CAGGTGCAGGGTTGACTCTTT-3′;
rat atrogin-1, 5′-AGATCCGCAAGCGATTGATC-3′—5′-TTGGGTAACATCGCACAAGC-3′;
rat MuRF1, 5′-TGAGCCACAAGTTTGACGC-3′—5′-CGATGAAGTCCAGCTTCTCCT-3′;
and β-actin, 5′-ATCGCTGACAGATCGAGAA-3′—5′-TAGAGCCACAATCCACACAG-3′.

The relative expression of each mRNA was calculated by the ΔΔCt method (where ΔΔCt is the value obtained by subtracting the Ct value of β-actin mRNA from the Ct value of the target mRNA), as employed in previous studies (19). Specifically, the amount of target mRNA relative to β-actin mRNA is expressed as 2−ΔΔCt. Data are expressed as the ratio of the target mRNA to β-actin mRNA. Studies were conducted in duplicate.

**Statistical methods.** Tests for statistical significance were performed using Statistical Analysis Software (ver-
Values for each group are presented as means ± standard error (SE). Differences among groups were analyzed by two-way analysis of variance (ANOVA), when appropriate, followed by Tukey’s post-hoc test. Values of \( p < 0.05 \) were considered statistically significant.

**RESULTS**

The \( T_b \), plasma MeHis and creatinine concentrations of rats 3 h after the start of test solutions infusion are shown in Fig. 1A–C. \( T_b \) tended to be higher by 0.4˚C on average in the conscious group given AA (Nor-AA group) than in the conscious group given saline (Nor-saline group), but no significant difference was found (Fig. 1A). In contrast, \( T_b \) was significantly lower in the anesthetized group compared to that of conscious rats. \( T_b \) was significantly higher in the anesthetized group given AA (Ane-AA group, 33.5 ± 0.4˚C; \( n = 10 \)) than in the anesthetized group given saline (Ane-saline group, 32.0 ± 0.8˚C; \( n = 9 \)). Plasma MeHis concentrations did not differ between Nor-saline and Ane-saline groups (Fig. 1B). By contrast, plasma MeHis concentrations were higher in AA groups compared to saline groups under both anesthetized and conscious conditions \( (p < 0.05) \). Furthermore, increments of plasma MeHis levels were more prominent in the Ane-AA group than in the Nor-AA group, with a significant difference observed between these groups. Plasma creatinine concentrations did not differ among groups (Fig. 1C).

Figure 2A and B shows the correlation between rectal temperature and plasma MeHis concentrations of conscious and anesthetized rats 3 h after the start of test solutions infusion, respectively. Higher plasma MeHis concentrations were associated with higher rectal temperature under both the anesthetized \( (r = 0.66, p < 0.05, n = 19) \) and conscious conditions \( (r = 0.67, p < 0.05, n = 20) \).

Figure 3 shows the mRNA expression of atrogin-1 (A and D), MuRF1 (B and E) and ubiquitin (C and F) in gastrocnemius (A, B and C) and soleus (D, E and F) muscles in conscious and anesthetized rats. These values were examined in rats after 3-h administration with either an AA mixture (AA: closed bars) or saline (SAL: open bars), either with the anesthetic propofol (right: Ane) or without anesthetic (left: Nor). Values represent mean ± SE \( (n = 10) \). Means not sharing a superscript are significantly different according to the Tukey-Kramer multiple comparisons test \( (p < 0.05) \).
nemius muscle was significantly affected by anesthetic treatment. The mRNA of atrogin-1 in soleus under both conditions and of atrogin-1 and ubiquitin in gastrocnemius muscle under the conscious condition became significantly lower in the AA group than in the saline group (p<0.05).

DISCUSSION

The present study revealed that plasma MeHIs concentrations were higher in the rats given AA than in those given saline under both conscious and anesthetized conditions. Furthermore, plasma MeHIs levels correlated positively with Tb of rats under both conditions. On the other hand, AA administration decreased most, if not all, mRNA expression of ubiquitin, atrogin-1 and MuRF1 gene in skeletal muscle compared to Sal administration.

Myofibrillar protein breakdown is often examined using urinary MeHis excretion. However, collecting urine is generally difficult in anesthetized rats due to muscular relaxation in the urinary bladder resulting from anesthesia. Nagasawa et al. reported that the amount of MeHIs released from isolated muscles into medium during a 2-h incubation period increases with starvation and corresponds to plasma MeHIs concentrations (12). In cases where the experimental period is relatively short, as in the present study, and urine collection is difficult, discussion of plasma concentrations as an index of myofibrillar protein breakdown appears appropriate. In this study, plasma creatinine concentrations did not differ among groups, strongly suggesting that neither anesthetic nor the test solution affects the filtration ratio of substances like MeHIs, which are not reabsorbed in the renal tubules. Plasma MeHIs concentrations would thus reflect the amount of MeHIs released from muscles.

Of note is the finding that AA administration enhanced myofibrillar protein breakdown under both anesthetized and conscious conditions. Results from several previous reports support the present finding about enhanced myofibrillar proteolysis following AA administration. Svanberg et al. noted that alterations in MeHIs release from human arm and leg muscle tissues in response to increasing doses of AA mixture infusion showed an increasing trend (20). Furthermore, urinary MeHIs output was elevated in response to increased dietary protein levels in rats under conditions of restricted food intake (21). Conversely, AAs, particularly branched chain AAs, are well known to inhibit myofibrillar proteolysis (22–24). Interestingly, Omstedt et al. observed that the better the quality of dietary protein that should result from differences in AA composition, the higher the MeHIs excretion in rats (25). Therefore, the composition of AA given may account for previous discrepancies in AA effects on myofibrillar proteolysis. In fact, we used a commercially available AA mixture (Table 1) as Svanberg et al. did (20). Another plausible explanation is that insufficiency of non-protein energy might have caused the increment of plasma MeHIs concentrations in this study. Hypocaloric AA mixture infusion leads to elevated urinary MeHis excretion compared to sufficient caloric nutrition containing the same amount of nitrogen (26).

Moreover, the urinary MeHis excretion in response to dietary protein ingestion is known to be positively correlated with net protein utilization in rats (25). This implies that AAs administered and AAs liberated from the proteolyzed muscle tissue were used for protein synthesis. We have previously reported that muscle protein synthesis is stimulated in both anesthetized and conscious rats given AA mixture under a similar experimental design (11). Taken together, the present finding that plasma MeHIs concentrations were increased by AA administration would thus not reflect any specific effect on myofibrillar protein breakdown, but could be the result of a general increase in muscular protein turnover.

Protein turnover is a substrate cycle that accounts for roughly 20% of thermo-neutral heat production (27). Increases in metabolic heat production by elevated protein turnover would be attributable to either incorporation of ingested or degraded AA into protein or their direct oxidation, as both reactions are accompanied by increases in energy expenditure (10). For example, it is known that in ad lib.-fed chicks exposed to 22°C, whole body protein synthesis and degradation were increased compared with those exposed to 30°C (28), possibly leading to the prevention of Tb decline. Therefore, one might predict that increased rates of protein turnover after AA or protein ingestion would contribute to elevated thermogenesis. In fact, plasma MeHis concentrations that would reflect increased protein turnover as discussed above displayed good correlations with rectal temperature under both anesthetic and conscious conditions. Furthermore, it is well known that AA administration increases oxygen consumption and elevates Tb under both conscious and anesthetized conditions (1–3, 6). These findings therefore indicate that increased protein turnover in rats given AA contributes to increased metabolic heat production, resulting in elevated Tb.

The present study also revealed that anesthetic administration alone did not affect plasma MeHIs concentrations. Anesthetics generally induce a decrease in Tb, as seen in anesthetized rats in the present study, and Tb of anesthetized rats should be above the thermoregulatory threshold for thermogenesis (9). Thermogenic responses against Tb decline might therefore not have been evoked, which would be different from the above-mentioned thermoregulatory response (increased protein turnover) to cold under the conscious condition (28). This is supported by the finding that urinary MeHis excretion after cardiac surgery did not differ between patients randomized to intraoperative blood temperatures of 28 or 20°C (29). In addition, myofibrillar protein degradation (MeHIs release) in white or red muscles incubated at different temperatures is unaffected by temperature (30). Taken together, these findings may imply that hypothermic contributions by anesthetic treatment to the regulation of myofibrillar
protein degradation are minimal. This suggests that changes in myofibrillar protein turnover offer a determinant of Tb modification by AA administration and that Tb does not adjust myofibrillar protein turnover.

We showed herein that AA administration down-regulates mRNA expressions of several ubiquitin-proteasome-proteolytic pathway components in skeletal muscle of both anesthetized and conscious rats. This observation is consistent with in vivo data showing several AAs, particularly leucine, isoleucine, glycine, suppress the expression of ubiquitin-proteasome-proteolytic related genes in skeletal muscle (23, 31, 32). Furthermore, activation of insulin signaling in skeletal muscle, particularly phosphorylation of protein kinase B (PKB) and forkhead transcription factor Foxo3, in general, suppresses mRNA expression of atrogin-1 and MuRF1 genes (33–36). In our previous study, PKB phosphorylation was clearly observed in both anesthetized and conscious rats given amino acids (11). This suggests that the decreases in mRNA expression of ubiquitin-proteasome-proteolytic related genes by AA administration likely result from the activation of insulin signaling, although Foxo3 phosphorylation has not been determined. However, decreases in mRNA expression of ubiquitin-proteasome-proteolytic related genes in skeletal muscle in this study did not exactly reflect plasma insulin levels (11). Although the reasons for this remain unclear, anesthetic treatment may affect unknown factors that prevent the insulin signaling pathway in the ubiquitin-proteasome pathway.

The ubiquitin-proteasome-proteolytic pathway among several proteolytic pathways is up-regulated in various catabolic states that cause muscle proteolysis (14, 15, 33, 34). Suppression of mRNA expression of the ubiquitin-proteasome-proteolytic related genes, in general, prevents catabolic response produced by catabolic insult (16, 17, 36). In contradiction to these finding, our data and other studies suggest that atrophic response in skeletal muscle can occur independently of ubiquitin-proteasome-proteolytic related gene expression (37–39). We speculate on some possible explanations for the dissociation between the effects of AA administration on the expression of mRNA associated with the ubiquitin-proteasome-proteolytic pathway and myofibrillar proteolysis. First, levels of mRNA expression of the genes and myofibrillar proteolysis were determined under non-stressed conditions in the current experiment. This implies that the role of gene expression of the ubiquitin-proteasome pathway in myofibrillar proteolysis is important just in cases where catabolic insult occurs. Second, other components involving the proteolytic pathway are actually rate-limiting, despite the fact that the activation of insulin signaling is prominent in rats given AAs (11). In fact, microarray profiling of human skeletal muscle revealed that increased plasma insulin concentrations elevate expression of 17 mRNAs for ubiquitin-conjugating enzymes and 11 mRNAs for the proteosome components (40). Third, down-regulation in the two ligases, especially MuRF1 in the current study, was not enough to decrease myofibrillar proteolysis. Mice lacking both atorogin-1 and MuRF1 exhibit the greatest protection from denervation-induced muscle loss compared to mice lacking only one of these genes (41).

In conclusion, the results shown herein strongly suggest that AA administration for 3 h enhances myofibrillar protein degradation, which is reflected in Tb modification in both anesthetized and conscious rats. Taken together with our previous finding that AA administration enhances muscle protein synthesis in both situations, the current results imply that changes in myofibrillar protein turnover offer a determinant of Tb modification by AA administration. In addition, our data suggest that decreases in ubiquitin-proteasome-proteolytic related gene expression examined herein do not necessarily predict a coordinate change in myofibrillar proteolysis. Hence, the precise mechanisms regulating the myofibrillar protein degradation by AA mixture administration remain yet to be determined.

Acknowledgments
We gratefully acknowledge the expert technical assistance of Mieko Fujichuki.

REFERENCES
1) Brundin T, Wahren J. 1994. Influence of protein ingestion on human splanchnic and whole-body oxygen consumption, blood flow, and blood temperature. Metabolism 43: 626–632.
2) Brundin T, Wahren J. 1994. Effects of i.v. amino acids on human splanchnic and whole body oxygen consumption, blood flow, and blood temperatures. Am J Physiol 266: E396–402.
3) Selldén E, Brundin T, Wahren J. 1994. Augmented thermic effect of amino acids under general anaesthesia: a mechanism useful for prevention of anaesthesia-induced hypothermia. Clin Sci (Lond) 86: 611–618.
4) Selldén E, Lindahl SG. 1999. Amino acid-induced thermogenesis reduces hypothermia during anesthesia and shortens hospital stay. Anesth Analg 89: 1551–1556.
5) Umenai T, Nakajima Y, Sessler DI, Taniguchi S, Yaku H, Mizobe T. 2006. Perioperative amino acid infusion improves recovery and shortens the duration of hospitalization after off-pump coronary artery bypass grafting. Anesth Analg 103: 1386–1393.
6) Kasai T, Nakajima Y, Matsukawa T, Ueno H, Sunaguchi M, Mizobe T. 2003. Effect of preoperative amino acid infusion on thermoregulatory response during spinal anaesthesia. Br J Anaesth 90: 58–61.
7) Sessler DI. 1997. Mild perioperative hypothermia. N Engl J Med 336: 1730–1737.
8) Kurz A, Sessler DI, Lenhardt R. 1996. Perioperative normothermia to reduce the incidence of surgical-wound infection and shorten hospitalization. Study of Wound Infection and Temperature Group. N Engl J Med 334: 1209–1215.
9) Lenhardt R, Marker E, Goll V, Tschernich H, Kurz A, Sessler DI, Narzt E, Lackner F. 1997. Mild intraoperative hypothermia prolongs postanesthetic recovery. *Anesthesiology* 87: 1318–1323.

10) Flatt JP. 1978. The biochemistry of energy expenditure. In: Recent Advances in Obesity Research (Bray G, ed.), Vol 2, Chapt 22, p 211–218. Newman, London.

11) Yamaoka I, Doi M, Nakayama M, Ozeki A, Mochizuki S, Sugahara K, Yoshizawa F. 2006. Intravenous administration of amino acids during anesthesia stimulates muscle protein synthesis and heat accumulation in the body. *Am J Physiol Endocrinol Metab* 290: E882–888.

12) Nagasawa T, Yoshizawa F, Nishiizawa N. 1996. Plasma Ntua-methylhistidine concentration is a sensitive index of myofibillary protein degradation during starvation in rats. *Biochi Biotechnol Biochem* 60: 501–502.

13) Rao DR, Elayskardi B, Pulusani SR, Chawan CB. 1982. Plasma 3-methyl histidine as an index of dietary protein quality in rats. *Nutr Rep Int* 26: 355–364.

14) Lecker SH, Solomon V, Mitch WE, Goldberg AL. 1999. Muscle protein breakdown and the critical role of the ubiquitin-proteasome pathway in normal and disease states. *J Nutr* 129: 2278–2378.

15) Roos-Mattjus P, Sistonen L. 2004. The ubiquitin-proteasome pathway. *Am J Physiol* 313: 285–295.

16) Frost RA, Nyström GJ, Jefferson LS, Lang CH. 2007. Hormone, cytokine, and nutritional regulation of sepsis-induced increases in atrogin-1 and MufR1 in skeletal muscle. *Am J Physiol Endocrinol Metab* 292: E501–512.

17) Nakashima K, Yakabe Y, Yamazaki M, Abe H. 2006. Effects of fasting and refeeding on expression of atrogin-1 and Akt/FOXO signaling pathway in skeletal muscle of chicks. *Biochi Biotechnol Biochem* 70: 2775–2784.

18) Larsen AE, Tunstall RJ, Carey KA, Nicholas G, Kambadur R, Crowe TC, Cameron-Smith D. 2006. Actions of short-term fasting on human skeletal muscle myogenic and atrogenic gene expression. *Ann Nutr Meta* 50: 476–481.

19) Nishimura M, Naito S. 2008. Tissue-specific mRNA expression profiles of human soleus carrier transport superfamilies. *Drug Metab Pharmacokinet* 23: 22–44.

20) Svanberg E, Möller-Lösvick AC, Matthews DE, Körner U, Andersson M, Lundhölm K. 1996. Effects of amino acids on synthesis and degradation of skeletal muscle proteins in humans. *Am J Physiol* 271: E718–724.

21) Nishizawa N, Shimo M, Hareyama S, Funabiki R. 1977. Fractional catabolic rates of myosin and actin estimated by urinary excretion of Ntua-methylhistidine: the effect of dietary protein level on catabolic rates under conditions of restricted food intake. *Br J Nutr* 37: 345–353.

22) Nakashima K, Ishida A, Yakabe Y, Yamazaki M, Abe H. 2006. Effects of orally administered amino acids on myofibillary proteinolyis in chicks. *Biochi Biotechnol Biochem* 70: 1975–1978.

23) Nakashima K, Ishida A, Yamazaki M, Abe H. 2005. Leucine suppresses myofibillary proteinolysis by down-regulating ubiquitin-proteasome pathway in chick skeletal muscles. *Biochi Biotechnol Res Commun* 336: 660–666.

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

25) Omstedt PT, Kihlberg R, Tingvall P, Shenkin A. 1978. Effect of dietary protein on urinary excretion of 3-methylhistidine in rat. *J Nutr* 108: 1877–1882.

26) Karlsson T, Stjernström H, Wilkund L, Groth T, Hjelm M. 1994. The effects of different intraoperative feeding regimens on the rates of synthesis of alpha1-antitrypsin after surgery. *Clin Nutr* 13: 90–97.

27) Newsholme EA. 1987. Substrate cycles and energy metabolism: their biochemical, physiological and pathological importance. In: Energy Metabolism of Farm Animals (Moe PW, Tynell HE, Reynold P, eds), European Association for Animal Production Publication No. 32. p 174–186. Rowman, Littlefield, Totowa, NJ.

28) Aoyagi Y, Tasaki I, Okamura J, Muramatsu T. 1988. Effect of low ambient temperature on protein turnover and heat production in chicks. *Comp Biochem Physiol A* 89: 433–436.

29) Taggart DP, McMillan DC, Preston T, Shenkin A, Wheatley DJ, Burns HJ. 1991. Effect of surgical injury and intraoperative hypothermia on whole body protein metabolism. *Am J Physiol* 260: E118–125.

30) Hall-Angerås M, Angerås U, Hasselgren PO, Fischer JE. 1990. Effects of elevated temperature on protein breakdown in muscles from septic rats. *Am J Physiol* 258: C589–592.

31) Nakashima K, Yakabe Y, Ishida A, Katsumata M. 2007. Effects of orally administered glycine on myofibrillar proteinolyis and expression of proteolytic-related genes of skeletal muscle in chicks. *Amino Acids* 35: 451–456.

32) Nakashima K, Yakabe Y, Ishida A, Yamazaki M, Abe H. 2007. Suppression of myofibrillar proteinolyis in chick skeletal muscles by alpha- ketoaspartate. *Amino Acids* 33: 499–503.

33) Lee SW, Dai G, Hu Z, Wang X, Du J, Mitch WE. 2004. Regulation of muscle protein degradation: Coordinated control of apoptotic and ubiquitin-proteasome systems by phosphatidylinositol 3 kinase. *J Am Soc Nephrol* 15: 1357–1545.

34) Sandri M, Sandri C, Gilbert A, Skulac E, Calabria B, Picard A, Walsh K, Schiaffino S, Lecher SH, Goldberg AL. 2004. Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell 117*: 399–412.

35) Stütz TN, Drujan D, Clarke BA, Panaro F, Timofeyva Y, Cai D, Frantz JD, Tawa NE Jr, Melendez P A, Oh BC, Lidov HG, Gonzalez M, Vancopoulos GD, Glass DJ. 2004. The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. *Mol Cell* 14: 395–403.

36) Saccheck JM, Ohtsuka A, McLary SC, Goldberg AL. 2004. IGF-1 stimulates muscle growth by suppressing protein breakdown and expression of atrophy-related ubiquitin ligases, atrogin-1 and MuRF1. *Am J Physiol* 287: E591–E601.

37) Vary TC, Frost RA, Lang CH. 2008. Acute alcohol intoxication increases atrogin-1 and MuRF1 mRNA without increasing proteolysis in skeletal muscle. *Am J Physiol Regul Integr Comp Physiol* 294: R1777–1789.

38) Cai D, Frantz JD, Tawa NE Jr, Melendez PA, Oh BC, Lidov HG, Hasselgren PO, Frontera WR, Lee J, Glass DJ, Shoelson SE. 2004. IKKbeta/NF-kappab activation causes severe muscle wasting in mice. *Cell 119*: 285–298.

39) Fareed MU, Evenson AR, Wei W, Mencioni M, Pouylin V, Petkova V, Pignol B, Hasselgren PO. 2006. Treatment of rats with calpain inhibitors prevents sepsis-induced muscle proteolysis independent of atrogin-1/MAFbx.
and MuRF1 expression. Am J Physiol Regul Integr Comp Physiol 290: R1589–1597.

40) Rome S, Clément K, Rabasa-Lhoret R, Loizon E, Poitou C, Barsh GS, Riou JP, Laville M, Vidal H. 2003. Microarray profiling of human skeletal muscle reveals that insulin regulates approximately 800 genes during a hyperinsulinemic clamp. J Biol Chem 278: 18063–18068.

41) Bodine SC, Laires 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.