

Note

## Suppression of Myofibrillar Protein Degradation after Refeeding in Young and Adult Mice

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**Summary** A diet containing adequate amounts of protein rapidly suppresses myofibrillar protein degradation after refeeding in young rats and mice. However, it is unclear whether this suppression is seen in adult animals. This study was undertaken to compare dietary protein-induced suppression of myofibrillar protein degradation in young and adult mice. Reductions in rates of myofibrillar protein degradation measured by *N*-methylhistidine (MeHis) released from the isolated extensor digitorum longus muscle were found at 4 to 7 h after refeeding in both young (7-wk-old) and adult (8-mo-old) mice, indicating that the response time of feeding-induced suppression of myofibrillar protein degradation was the same. When young (8-wk-old) mice were fed a 20% casein diet (20C) for 1 h after 18 h starvation, the rate of myofibrillar protein degradation was significantly decreased at 4 h after refeeding; however, mice fed a 10% casein diet (10C), 5% casein diet (5C), or protein-free diet (0C) did not show suppression of myofibrillar protein degradation. Adult (8-mo-old) mice fed 20C or 10C showed a reduction in the rate of MeHis release. The plasma concentration of leucine in young mice was only higher when they were fed 20C. Adult mice fed 20C or 10C showed higher plasma concentrations of leucine. These results suggest that postprandial suppression of myofibrillar protein degradation occurs in adult mice as in young mice, but the adult mice respond to a lower amount of dietary casein compared to the young mice.

**Key Words** protein turnover, myofibrillar protein, dietary protein, 3-methylhistidine

Aging and many catabolic diseases are accompanied by muscle wasting due to a decreased rate of synthesis and an increased rate of degradation of skeletal muscle, particularly myofibrillar protein (1–3), and these changes in synthesis and degradation accelerate further catabolic disorders. It is therefore important to maintain muscle mass in aging and diseases by regulating the synthesis and degradation of muscle protein. Numerous studies have investigated the factors regulating muscle protein turnover including hormones, cytokines, and amino acids (2, 4, 5). Long-term starvation causes loss of muscle mass by both reducing the rate of protein synthesis and increasing the rate of protein degradation (6). Refeeding immediately stimulates muscle protein synthesis due to the change in translational activity (7, 8). However, the changes in the degradation rate of muscle protein in a short period after refeeding remain to be elucidated. We have demonstrated postprandial suppression of myofibrillar protein degradation in young mice and rats (8, 9), but the suppression was not seen in rats fed a protein-free diet (8). These results suggest that dietary protein and specific amino acids are important factors in the suppression of

myofibrillar protein degradation.

It is unclear that the postprandial suppression of myofibrillar protein degradation is found in adult and older animals, since metabolic changes are greater in younger animals. Therefore, it is important to know whether the suppression occurs in adult and older animals. If dietary protein causes suppression of myofibrillar protein degradation at all ages, we can regulate muscle wasting during aging and diseases. In this experiment, we compared the suppression of myofibrillar protein degradation in young and adult mice fed diets of differing protein content.

### Materials and Methods

*Animal preparation and experimental protocol.* In Experiment 1, 20 male 5-wk-old and 20 male 8-mo-old ICR mice obtained from Clea Japan Inc. (Tokyo, Japan) were used. The animals were individually housed in stainless steel wire cages and maintained at 22°C and 55% relative humidity on a 12 h light–dark cycle (6 AM–6 PM). They were allowed free access to water and a 20% casein diet (10) for 3 d. To accustom them to feeding within a short period, the diet was given at 10 AM and was withdrawn at 10 PM for 3 d, then was given at 10 AM and withdrawn at 7 PM for 3 d, and

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finally given at 10 AM and withdrawn at 4 PM for 6 d. At the end of this feeding period, the mice in each age group were randomly assigned to 4 groups ( $n=5$  in each group) and starved for 18 h. The mice in one group were sacrificed before refeeding commenced. The young and adult mice in the remaining three groups were refed a 20% casein diet for 1 h and were sacrificed at 4, 7 and 13 h after refeeding. All mice were anesthetized with diethylether. Blood was collected from the jugular vein to obtain plasma. The plasma was frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until analysis. The extensor digitorum longus (EDL) muscle, a typical white fast-twitch muscle was removed from the right leg. In Experiment 2, 30 6-wk-old and 30 8-mo-old ICR mice were obtained from Clea Japan Inc. The feeding protocol was the same as in Experiment 1. At the end of the feeding period, the mice in each age group were randomly assigned to 5 groups ( $n=6$  in each group) and starved for 18 h. One group of mice (Starved) was sacrificed before refeeding. The mice in remaining groups were refed a protein-free diet (0C), a 5% casein diet (5C), a 10% casein diet (10C) or a 20% casein diet (20C), respectively, for 1 h, and were sacrificed at 4 h after refeeding. Blood and the EDL muscle were removed as the same manner as in Experiment 1. The animal care protocols in this study were approved by the Iwate University Animal Research Committee, and the Iwate University Guidelines for Animal Experimentation were followed.

**Measurement of myofibrillar protein degradation.** To measure the rate of myofibrillar protein degradation directly, we incubated the isolated EDL muscles in Krebs-Ringer bicarbonate buffer containing 10 mmol/L glucose under 95%  $\text{O}_2$ -5%  $\text{CO}_2$  at  $37^{\circ}\text{C}$  for 2 h following a 30-min preincubation at  $37^{\circ}\text{C}$  (11).  $N^7$ -Methylhistidine (3-methylhistidine, MeHis) concentration in the buffer after incubation was measured by the HPLC method after derivatization of fluorescamin by treatment with perchloric acid and heat (12).

**Plasma measurement.** The plasma concentration of leucine was measured with an automatic amino acid analyzer (AminoTac, JLC-500/V, JEOL, Tokyo, Japan) following a sulfosalicylic acid treatment (final 1.5%).

**Statistical analysis.** Data are expressed as means  $\pm$  SE. Comparisons between starved level and feeding level within each age group were carried out using the two-tailed unpaired  $t$  test. Differences were considered significant at  $p < 0.05$ .

## Results and Discussion

The flooding dose of the isotope method can measure the rate of protein synthesis, and the rate of protein degradation is calculated by the difference between the rate of synthesis and growth (13). However, this method cannot evaluate an acute change in the rate of degradation after feeding. The arterial-venous difference in concentrations of amino acids can be used to determine the rate of muscle protein degradation (5), although this method is difficult to apply for small animals. In the present study, we measured the rate of

MeHis release from EDL muscle incubating in physiological buffer for 2 h. MeHis is not reutilized for protein synthesis and is not metabolized in muscle cells (14). This method can evaluate the acute nutritional and physiological changes in the myofibrillar protein degradation rate of small rats (11). However, when this method is used, the isolated muscle must be thin, such as the EDL and soleus muscles, which weigh less than 50 mg, because amino acid is not released accurately from muscle cells into the incubation buffer when more than 50 mg of muscle tissue is used (11). Therefore, we used young rats with body weights less than 100 g in the previous studies (9, 15). Svanverg et al. (16) have demonstrated that an isolated EDL muscle of adult mouse can be used as a tool to measure the rate of muscle protein degradation. They measured the synthesis rate by incorporation of radio-labeled phenylalanine into the isolated mouse EDL muscle from the incubation buffer and measured the degradation rate by determining the amount of tyrosine released from the EDL muscle. In Experiment 1, the body weights of young and adult mice were  $31 \pm 2$  g and  $46 \pm 3$  g (mean  $\pm$  SE), respectively. The weights of their EDL muscles were 15–20 mg, which was thin enough to allow the measurement of amino acid released from the isolated muscles. We could measure MeHis released from mouse EDL muscle using this method for the first time.

In young rats, reduction of myofibrillar protein degradation was found from 4 to 7 h after refeeding (9). Therefore, we first examined the time course of the rate of myofibrillar protein degradation after refeeding in young and adult mice. In young mice, the rate of MeHis release from EDL muscle decreased from 4 h after refeeding, and the decreased rate was maintained until 7 h. The rate then increased to the level of starved mice (Fig. 1). These changes in the rate of MeHis release from the isolated muscle of young mice were similar to those demonstrated in our previous studies using young rats (9), but the extent of reduction was greater than that in

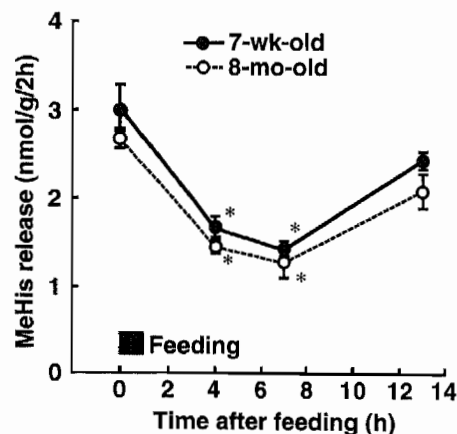


Fig. 1. Changes in  $N^7$ -methylhistidine (MeHis) released from extensor digitorum longus muscle of young (7-wk-old) and adult (8-mo-old) mice. Results are expressed as mean  $\pm$  SE ( $n=5$ ). \* $p < 0.05$  vs starvation (0 h).

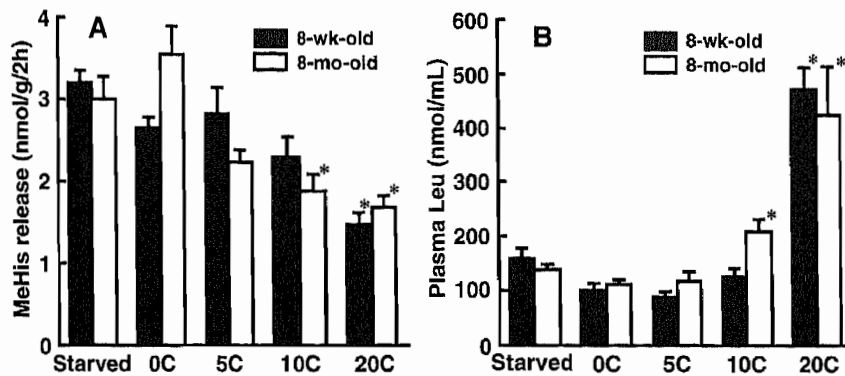


Fig. 2.  $N^m$ -methylhistidine (MeHis) released from extensor digitorum longus muscle (A) and plasma concentration of leucine (B) in young (8-wk-old) and adult (8-mo-old) mice fed a diet containing different amount of casein. Results are expressed as mean  $\pm$  SE ( $n=6$ ). \*  $p<0.05$  vs starvation (0 h).

rats. The adult mice showed the same pattern and extent of suppression of myofibrillar protein degradation as young mice did. Thus, suppression of myofibrillar protein degradation after refeeding is also demonstrated in adult mice.

In adult and older rats, muscle protein synthesis can be stimulated by diet as it is in young rats. Dardevert et al. (17, 18) have shown that a leucine-supplemented meal stimulates muscle protein synthesis rate in 8- and 22-mo-old rats. Mosoni et al. (19) have reported that the rate of muscle protein synthesis is increased by refeeding after starvation in 12- and 24-mo-old rats. In spite of protein synthesis, the responses of muscle protein degradation to feeding in adult and older animals are unclear. It is possible that young animals have a higher sensitivity to diet than adult and older animals do. Therefore, we next examine the response of myofibrillar protein degradation to dietary protein level.

In Experiment 2, the body weights of young and adult mice were  $30 \pm 1$  g and  $46 \pm 3$  g, respectively (mean  $\pm$  SE). The food intakes of young and adult mice were  $1.4 \pm 0.7$  g and  $1.3 \pm 0.5$  g, respectively (mean  $\pm$  SE), with no significant differences among diet groups. In young mice, the suppression of MeHis release was only found after feeding of 20C, but in adult mice it was found after feeding of both 10C and 20C (Fig. 2A). Thus, adult mice appear to respond to a lower content of protein in the diet. Volpi et al. (20, 21) have shown that oral amino acids stimulate muscle protein synthesis in the elderly human. Leucine is reported to be an amino acid stimulating muscle protein synthesis (22). Dardevet et al. (17, 18) have demonstrated that the effect of leucine on muscle protein synthesis can be demonstrated in adult and older rats as well as young rats. We have shown that oral administration of leucine suppresses myofibrillar protein degradation (15). Therefore, the plasma concentration of leucine may be a key factor for suppression of myofibrillar protein degradation as well as stimulation of protein synthesis. The plasma concentration of leucine increased in young mice fed 20C, while it did not increase in those fed 10C, 5C or 0C (Fig. 2B). The plasma concentration of leucine increased in adult mice fed 10C or 20C. The suppres-

sion of myofibrillar protein degradation correlated well with the increase in plasma concentration of leucine (the correlation coefficient values were  $-0.683$  for young mice and  $-0.513$  for adult mice,  $p<0.05$ ). In young mice, low protein content in the diet did not increase plasma amino acid concentration to inhibit myofibrillar protein degradation, whereas in adult mice even a 10% protein diet induced an increase in plasma amino acid concentration, which might be a signal to suppress degradation. Imai et al. (23) have demonstrated that serine dehydratase activity in adult rat liver increases with the feeding of a lower-protein diet, although that of a young rat is increased only by a higher-protein diet. Therefore, adult and possibly older animals can respond to a lower protein content in the diet, which might reflect a lower protein requirement.

Insulin is also a key factor in the regulation of muscle protein synthesis and degradation (4). It is known that age-related glucose intolerance is often associated with insulin resistance (24). Furthermore, other hormones related to muscle protein turnover alter with age (25). Therefore, the physiological changes with age may influence the difference of response to myofibrillar protein degradation after feeding.

In conclusion, we have shown that suppression of myofibrillar protein degradation after refeeding occurs in young and adult mice, but the response to dietary protein differs between age groups. Our results strongly suggest that dietary protein is an important factor in the regulation of myofibrillar protein turnover, and the present findings show beneficial effects on the maintenance of muscle mass for adults and the elderly.

#### REFERENCES

- 1) Bross R, Storer T, Bhasin S. 1999. Aging and muscle loss. *Trends Endocrinol Metab* 10: 194-198.
- 2) Garlick PJ, McNurlan MA, Bark T, Lang CH, Gelato MC. 1998. Hormonal regulation of protein metabolism in relation to nutrition and disease. *J Nutr* 128: 356S-359S.
- 3) Welle S. 1999. Human Protein Metabolism. p 196-227. Springer-Verlag, New York.
- 4) Rooyackers OE, Nair KS. 1997. Hormonal regulation of human muscle protein metabolism. *Annu Rev Nutr* 17:

- 457–485.
- 5) Volpi E, Kobayashi H, Sheffield-Moore M, Mittendorfer B, Wolfe RR. 2003. Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. *Am J Clin Nutr* **78**: 250–258.
  - 6) Li JB, Goldberg AL. 1976. Effects of food deprivation on protein synthesis and degradation in rat skeletal muscle. *J Physiol* **231**: 441–448.
  - 7) Yoshizawa F, Endo M, Ide H, Yagasaki K, Funabiki R. 1995. Translational regulation of protein synthesis in the liver and skeletal muscle of mice in response to refeeding. *J Nutr Biochem* **6**: 130–136.
  - 8) Yoshizawa F, Nagasawa T, Nishizawa N, Funabiki R. 1997. Protein synthesis and degradation change rapidly in response to food intake in muscle of food-deprived mice. *J Nutr* **127**: 1156–1159.
  - 9) Nagasawa T, Hirano J, Yoshizawa F, Nishizawa N. 1998. Myofibrillar protein catabolism is rapidly suppressed following protein feeding. *Biosci Biotechnol Biochem* **62**: 1932–1937.
  - 10) Reeves PG, Nielsen FH, Fahey GC. 1993. AIN-93 purified diets for laboratory rodents: final reports of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr* **123**: 1939–1951.
  - 11) Hasselgren P-O, James JH, Benson DW, Hall-Angerås M, Hiyama DT, Li S, Fischer JE. 1989. Total and myofibrillar protein breakdown in different type of rat skeletal muscle: effects of sepsis and regulation by insulin. *Metabolism* **38**: 634–640.
  - 12) Wassner SJ, Schlitzer JA, Li JB. 1980. A rapid, sensitive method for the determination of 3-methylhistidine levels in urine and plasma using high-performance liquid chromatography. *Anal Biochem* **104**: 284–289.
  - 13) Jahoor F, Zhang X-J, Baba H, Sakurai Y, Wolfe RR. 1992. Comparison of constant infusion and flooding dose techniques to measure muscle protein synthesis rate in dogs. *J Nutr* **122**: 878–887.
  - 14) Young VR, Munro HN. 1978. N<sup>3</sup>-Methylhistidine (3-methylhistidine) and muscle protein turnover. *Fed Proc* **37**: 2291–2300.
  - 15) 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.
  - 16) Svanberg E, Svaninger G, Soussi B, Lundholm K. 1999. Mouse extensor digitorum longus muscle preparation as a tool in nutrition research: A quantitative comparison to in vivo and cell culture experiments. *Nutrition* **15**: 200–207.
  - 17) Dardevet D, Sornet C, Balage M, Grizard J. 2000. Stimulation of in vitro rat muscle protein synthesis by leucine decreases with age. *J Nutr* **130**: 2630–2635.
  - 18) Dardevet D, Sornet C, Bayle G, Prugnaud J, Pouyet C, Grizard J. 2002. Postprandial stimulation of muscle protein synthesis in old rats can be restored by a leucine-supplemented meal. *J Nutr* **132**: 95–100.
  - 19) Mosoni L, Malmezat T, Valluy MC, Houlier ML, Attaix D, Mirand PR. 1999. Lower recovery of muscle protein lost during starvation in old rats despite a stimulation of protein synthesis. *Am J Physiol* **277**: E608–E616.
  - 20) Volpi E, Ferrando AA, Yeckel CW, Tipton KD, Wolfe RR. 1998. Exogenous amino acids stimulate net muscle protein synthesis in the elderly. *J Clin Invest* **101**: 2000–2007.
  - 21) Volpi E, Mittendorfer R, Wolf SE, Wolfe RR. 1999. Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction. *Am J Physiol* **277**: E513–E520.
  - 22) Anthony JC, Yoshizawa F, Anthony TG, Vary TC, Jefferson LS, Kimball SR. 2000. Leucine stimulates translation initiation in skeletal muscle of postabsorptive rats via a rapamycin-sensitive pathway. *J Nutr* **130**: 2413–2419.
  - 23) Imai S, Kanamoto R, Yagi I, Kotaru M, Sacki T, Iwami K. 2003. Response of the induction of rat liver serine dehydrogenase to changes in the dietary protein requirement. *Biosci Biotechnol Biochem* **67**: 383–387.
  - 24) Chang AM, Halter JB. 2003. Aging and insulin secretion. *Am J Physiol* **284**: E7–E12.
  - 25) Elmlinger MW, Dengler T, Weinstock C, Kuehnel W. 2003. Endocrine alternations in the aging male. *Clin Chem Lab Med* **41**: 934–941.