Myofibrillar Protein Catabolism is Rapidly Suppressed Following Protein Feeding

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The immediate response of protein degradation to food intake and the factors for its regulation in rat skeletal muscle were examined. The concentration of $N\text{-}$methylhistidine (MeHis) in serum and the rates of MeHis release from isolated soleus and extensor digitorum longus muscles were reduced in the period from 3 to 6 h after refeeding, indicating that the rate of myofibrillar protein degradation in the rat decreased immediately after refeeding. Changes in the serum concentration of insulin and corticosterone were not synchronized with those in the myofibrillar protein degradation. When rats were fed on a protein-free diet, no reduction of serum MeHis concentration or of the rate of MeHis release from isolated muscles after refeeding was apparent. Furthermore, there was a tendency toward suppressing myofibrillar protein degradation with a higher protein content of the diet. These results suggest that the suppression of myofibrillar protein degradation by food intake was regulated by dietary proteins.

Key words: protein degradation; myofibrillar protein; dietary protein; insulin; rat

Long-term starvation induces an increase in the protein degradation of skeletal muscle in human and laboratory animals.1-9 Rats subjected to refeeding have demonstrated a reduction in the rate of muscle protein degradation.1,3,4,6,9 This decrease in degradation due to refeeding is associated with changes in the plasma concentration of many hormones such as insulin, insulin-like growth factor-I (IGF-I), triiodothyronine ($T_3$), and growth hormones which regulate muscle protein turnover.10

In contrast to long-term starvation, the response of protein metabolism to brief starvation (less than 1 d) such as that in the postprandial state is not clear. In skeletal muscle, the rate of protein synthesis is stimulated by food intake after brief starvation.1,11-13 However, since there are no direct methods for measuring protein degradation in the acute period, the postprandial degradation rate of skeletal muscle protein has been measured in only few studies.1,13,14 The degradation rate of skeletal muscle protein can be measured by the urinary excretion of $N\text{-}$methylhistididine (MeHis).15 MeHis is localized in myofibrillar proteins, myosin and actin, but it is not reused for protein synthesis.15 Measuring the urinary excretion of MeHis does not detect acute changes in myofibrillar protein degradation, because the collection period for urine is usually 1 d. In contrast, the plasma MeHis concentration can reflect an acute change in the degradation of myofibrillar protein.7,16

Our previous study on mice has demonstrated that the plasma MeHis concentration decreased by 80% 3 h after food intake, and we suggested that the myofibrillar protein degradation was strongly and immediately suppressed by food intake.13 However, this dramatic reduction of plasma MeHis concentration may have been due to a recovery of kidney function or to an increased blood flow stimulated by food intake. The aim of the present study is to investigate whether the decreased MeHis concentration in blood could be seen in rats as we have observed in mice15 and to measure directly the degradation rate of myofibrillar protein after food intake. We also investigated the hormonal response and the effect of dietary protein on the reduction of postprandial protein degradation in skeletal muscle. Our results indicate that a dietary factor is associated with the reduction in muscle protein degradation after food intake.

Materials and Methods

Animals and diet. Experiment 1. Thirty male Sprague-Dawley rats of 3 wk of age were purchased from Nippon Clea (Tokyo, Japan). They were individually housed in stainless steel wire cages and maintained at 22°C and 55% relative humidity with a 12-h light-dark cycle (6:00-18:00). They were allowed free access to water and a 20% casein diet according to AIN76 for 3 d. To accent them to feeding within a short period, the daily feeding schedule was changed as follows: the diet was given from 10:00 to 22:00 for 3 d, then from 10:00 to 19:00 for 3 d, and finally from 10:00 to 16:00 for 6 d. At the end of the feeding period, the rats were starved for 18 h and refeed for 1 h. They were anesthetized by diethylether and killed at 1 (before feeding), 0, 3, 6 or 12 h after refeeding was finished. The abdomen was opened and blood was collected from the inferior vena cava to obtain serum. The soleus and extensor digitorum longus (EDL) muscles were removed from the right leg. Sera were frozen in liquid nitrogen and stored at $-80^\circ$C until analysis. The animal care protocol in this study was approved by Iwate University.

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Abbreviations: EDL, extensor digitorum longus; IGF-I, insulin-like growth factor-I; MeHis, $N\text{-}$methylhistidine; $T_3$, triiodothyronine
Animal Research Committee under Guidelines to Animal Experiments in Iwate University.

Experiment 2. Thirty male Sprague-Dawley rats of 3 wk of age were used. They were acclimatized to feeding for 6 h by the same method as that described for Experiment 1. After 18 h of starvation, 6 rats were killed, and 12 rats were fed on a protein-free diet for 1 h. The others were fed on a 20% casein diet for 1 h. They were all killed at 3 h and 6 h after refeeding was finished. Blood and muscle samples were then collected by the same methods as those described in Experiment 1.

Experiment 3. Thirty male Sprague-Dawley rats of 3 wk of age were used. They were acclimatized to feeding for 3 h by the same method as that described for Experiment 1, except for the final stage in which the diet was given from 10:00 to 13:00 for 6 d. After 21 h of starvation, 6 rats were killed, and the others were fed on a 0 (protein-free), 10, 20 or 40% casein diet for 1 h. This schedule enabled the rats to be fed on a greater amount of diet during the refeeding period. They were killed 3 h after refeeding was finished. The soleus and EDL muscles were isolated from the hind leg and used for measuring the rate of myofibrillar protein degradation.

Serum MeHis and creatinine. Serum was measured instead of plasma that had been measured previously, so that samples could also be assayed for their serum insulin and corticosterone concentrations. Deproteinized serum prepared by the previously described method was hydrolyzed with 6 M HCl (final concentration) at 110°C for 2 h to convert N-acetyl-MeHis to free MeHis. The hydrolyzate was evaporated to remove HCl and then dissolved in water. MeHis was measured by the previously described HPLC method after derivatization with o-phthalaldehyde. Serum creatinine was measured by the HPLC method of Murakita et al.

Muscle protein degradation. When the rate of protein degradation was measured directly, we incubated the isolated soleus and EDL muscles in a Krebs-Ringer bicarbonate buffer containing 10 mm glucose under 95% O₂-5% CO₂ at 37°C for 2 h after a 30-min pre-incubation at 37°C. The MeHis released into the incubation buffer was measured by the HPLC method after the derivatization of fluorescamine, treating with perchloric acid and heating.

Insulin and corticosterone assay. Insulin was measured by an enzyme-immunoassay with an insulin assay kit (Grazyme Insulin EIA Test, Wako Pure Chemicals, Osaka, Japan). The corticosterone concentration was determined by a radioimmunoassay with the Biotrack rat corticosterone [21] assay system (Amersham Life Science, UK).

Statistical analyses. Data analyses (GraphPad InStat Software version 2.03, 1995, San Diego, CA, U.S.A.) involved evaluating the means and SEM for each of the groups. An analysis of variance (ANOVA) was performed to determine whether there were significant (P < 0.05) differences among the groups. When ANOVA indicated any significant difference among the means, the Newman-Keuls multiple-comparison test was used to determine which means were significantly different.

Results

Experiment 1

The final body weight of the rats before food intake was 137.9 ± 6.3 g (mean ± SEM), and the food consumption was 7.4 ± 2.8 g (mean ± SEM). The weight of each muscle (soleus and EDL) before and after food intake was unchanged (data not shown).

The serum MeHis concentration before and after food intake is shown in Fig. 1. The MeHis concentration was decreased to 50% of the 18-h starved level within 3 h after refeeding. The MeHis concentration was maintained at this lower level until 6 h after food intake and then gradually increased to the pre-feeding level by 12 h after refeeding. The serum creatinine concentration was also decreased by the food intake (25% reduction) (Fig. 1).

The myofibrillar protein degradation rate was next measured directly by the rate of MeHis release from the soleus (slow-twitch) and EDL (fast-twitch) muscles during incubation into the buffer. Refeeding induced a decrease in MeHis release, which means that the rate of myofibrillar protein degradation decreased in both the soleus and EDL muscles, whereas the rate of MeHis release was higher in the soleus muscle than that in the EDL muscle (Fig. 2). The free MeHis concentrations in the soleus and EDL muscles were unchanged during the incubation period (data not shown). The rate of release was at a low level from 3 to 6 h after food intake (75% of the pre-feeding level) and increased to the pre-feeding level by 12 h after food intake.

The insulin concentration in the serum was dramatically increased by refeeding, and returned to the pre-
feeding level 3 h after food intake (Fig. 3). The corticosterone concentration in the serum decreased with food intake and remained at a low concentration until 12 h after refeeding.

**Experiment 2**

In this experiment, the rats were fed on a 20% casein diet or a protein-free diet for 1 h after food deprivation for 18 h. The final body weight before refeeding was 132.7 ± 7.5 g (mean ± SEM). The consumption of the 20% casein diet and protein-free diet was 6.9 ± 0.7 g and 6.1 ± 0.6 g (mean ± SEM), respectively, which is not significantly different ($P < 0.05$).

The serum MeHis concentration at 3 h after food intake was at the pre-feeding level in the rats which had been fed on the protein-free diet, whereas it significantly decreased in the rats which had been fed on the 20% casein diet (Fig. 4A). In this experiment, the serum creatinine concentration was not altered during the experimental period (data not shown). Figs. 4B and 4C show the MeHis release from the soleus and EDL muscles, respectively. The MeHis release from the soleus and EDL muscles of the rats fed on the 20% casein diet respectively decreased by 25% at 3 h and 6 h after food intake compared to the levels before refeeding. In contrast, the rate of MeHis release from both muscles in the rats which had been fed on the protein-free diet was not decreased by refeeding.

**Fig. 2.** Rates of $N^\omega$-Methylhistidine (MeHis) Release from Isolated Soleus and Extensor Digitorum Longus (EDL) Muscles.

Closed circles show the soleus muscle and open circles show the EDL muscle. Rats were starved for 18 h and refed for 1 h. The muscles were removed at $-1$, 0, 3, 6, and 12 h after the end of refeeding, and the isolated muscles were incubated at 37°C for 2 h in the buffer described in the Materials and Methods section. Each value is the mean ± SEM for 6 rats. Values with different letters are significantly different ($P < 0.05$).

**Fig. 3.** Serum Insulin and Corticosterone Concentrations in Rats before and after Refeeding for 1 h.

Closed circles show the insulin concentration and open circles show the corticosterone concentration. Rats were starved for 18 h and refed for 1 h. The serum was obtained from blood at $-1$, 0, 3, 6, and 12 h after the end of refeeding. Each value is the mean ± SEM of 6 rats. Values with different letters are significantly different ($P < 0.05$).

**Fig. 4.** Serum $N^\omega$-Methylhistidine (MeHis) Concentration and Rates of MeHis Release from Isolated Muscles after Refeeding with the Protein-Free Diet.

(A) Serum MeHis concentration, (B) MeHis release from the soleus muscle, and (C) MeHis release from the extensor digitorum longus muscle. Rats were starved for 18 h and refed a 20% casein diet or a protein-free diet for 1 h. The serum was obtained from blood and muscles were removed at $-1$, 3, and 6 h after the end of refeeding. Each value is the mean ± SEM of 6 rats. Values with different letters are significantly different ($P < 0.05$).
Myofibrillar Protein Degradation after Food Intake

![Graph showing MeHis release from different protein levels.](image)

**Fig. 5.** Rates of N\(^1\)-Methylhistidine (MeHis) Release from Isolated Soleus and Extensor Digitorum Longus (EDL) Muscles of Rats Fed on Different Levels of Dietary Protein.

Rats were starved for 21 h and refed with a 0, 10, 20 or 40% casein diet for 1 h. The muscles were removed 3 h after the end of refeeding, and the isolated muscles were incubated at 37°C for 2 h in the buffer described in the Materials and Methods section. Each value is the mean±SEM of 6 rats. Values with different letters are significantly different (P<0.05).

**Experiment 3**

We found a possibility that dietary protein reduced myofibrillar protein degradation in Experiment 2. Therefore, in this experiment, we examined the dose response of protein in the diet to myofibrillar protein degradation. The final body weight before refeeding was 162.1±2.4 g (mean±SEM). The food consumption of diet was not significantly different (P<0.05) among the diets, being 9.5±0.7 g for the refeeding period. The rate of MeHis release from both muscles tended to be suppressed depending upon the content of dietary protein (Fig. 5). When the rats were fed on the protein-free or 10% protein diet, the rate of MeHis release from the soleus and EDL muscles was not significantly different from that of the starved rats for 21 h (just before refeeding). In contrast, when the rats were fed on the 20% or 40% casein diet, the rate of MeHis release from the EDL muscle was significantly decreased compared to that from the starved animals. With the soleus muscle, the rats fed on the 40% casein diet showed a significant decrease in the rate of MeHis release from those starved for 21 h. The rate of MeHis release in Experiment 3 was slower than that in Experiments 1 and 2, probably due to the greater weight of the muscles (body weight).

**Discussion**

We have previously shown that the plasma MeHis concentration in mice decreased by 80% 3 h after refeeding.\(^1\) We demonstrated that the plasma MeHis concentration was correlated with the rate of myofibrillar protein degradation.\(^2\) Therefore, a reduction in the plasma MeHis concentration in mice after food intake suggests a decrease in the rate of myofibrillar protein degradation. There are two other possibilities to explain this reduction of MeHis concentration in plasma. One is a change in kidney function\(^1,21,22\) and the other is a change in the blood flow by food intake.\(^23\) In this study, a decrease in the serum MeHis concentration after food intake was observed in rats, similar to that which occurred in mice. In addition, the serum creatinine concentration was also decreased, although to a lesser extent than the MeHis concentration (Fig. 1). The observed decrease in serum creatinine concentration may indicate that food intake affected the kidney function or blood flow. Since the decrease in MeHis concentration 3 h after food intake (50%) was much greater than that in creatinine concentration (25%), we assume that the reduction of serum MeHis concentration must have been due to factors other than changes in the kidney function or blood flow, i.e., a decrease in the myofibrillar protein degradation.

The reduction in serum MeHis level after food intake suggests a decrease in myofibrillar protein degradation, although serum MeHis is derived from skeletal muscle tissue and non-skeletal muscle tissue such as gastrointestinal and skin.\(^24,29\) Therefore, we confirmed the decrease in the rate of myofibrillar protein degradation after food intake by direct measurement on isolated soleus and EDL muscles. Both muscles showed a reduction in the rate of MeHis release 6 h after food intake. These results strongly suggest that myofibrillar protein degradation was inhibited from 3 to 6 h after food intake. There are several studies indicating that refueling caused a suppression in the rate of myofibrillar protein degradation.\(^3,5,9\) However, in those studies, the rate of myofibrillar protein degradation was measured by a unit of one day, and not one hour. Li and Wassner\(^31\) have demonstrated that refueling for 24 h decreased the rate of MeHis release from hindquarters to a perfusate, suggesting an inhibition of myofibrillar protein degradation. Goodman and del Pilar Gomez\(^22\) have also shown, by using hindquarter perfusion of rat refed for 4-24 h, that the myofibrillar protein degradation was suppressed by food intake. Botbol and Scornik\(^26\) have also shown that refueling for 3.5 h reduced muscle protein degradation in mice. Therefore, refueling is an important signal for the inhibition of myofibrillar protein degradation. Our results demonstrate that the reduction of myofibrillar protein degradation began immediately after food intake with a maximal reduction 3 to 6 h after food intake.

The fact that the degradation rate of myofibrillar protein was immediately inhibited by food intake suggests the presence of some mediators that were induced by food intake. Refeeding caused an increase in the serum concentration of such substrates as glucose and amino acids,\(^2\) and changes occurred in the endocrine status,\(^20\) including a rise in insulin concentration which influences the protein turnover in skeletal muscle. Insulin is a well-known stimulator of the regulation of muscle protein synthesis\(^22,26,27\) and works by stimulating the activity of elongation factors in muscle cells in the postprandial state.\(^28\) Our previous work\(^13\) has indicated that the changes in plasma insulin concentration after food intake were synchronized with the changes in muscle protein synthesis as shown by polysome profiles. Moreover, since insulin reportedly had an inhibitory effect on mus-
cle protein degradation, it is likely that a higher level of insulin concentration in the serum after food intake may have inhibited protein degradation. However, when the rate of myofibrillar protein degradation was maintained at a low level from 3 to 6 h after refeeding (Fig. 2), the serum insulin concentration returned to the pre-feeding level. It has been reported that the effect of insulin on protein degradation was due to a reduction in the number of autophagosomes in cells or to a suppression of ubiquitin-ATP-dependent proteolysis, although the exact mechanism for insulin action against muscle protein degradation is still unclear. Therefore, insulin action may need several hours and could not immediately regulate myofibrillar protein degradation, unlike the stimulation of muscle protein synthesis.

Glucocorticoids are known to be factors which regulate the protein turnover in skeletal muscle. Glucocorticoids stimulate muscle protein degradation through an increase in the expression of ubiquitin and proteasome subunits in an ATP-ubiquitin-dependent proteolytic system. Wing and Goldberg and Medina et al. have demonstrated that the levels of polyubiquitin and proteasome mRNA were increased with food deprivation and decreased with refeeding. In the present experiment, the rats were starved for 18 or 21 h and refed for 1 h. Therefore, it is possible that glucocorticoids regulated postprandial myofibrillar protein degradation. We observed that the glucocorticoid (corticosterone) concentration was decreased by 50% 1 h after food intake began and that the concentration remained at this low level until 12 h after food intake, when the rate of myofibrillar protein degradation had returned to the pre-feeding level. This suggests that the decreased glucocorticoid concentration alone was unable to regulate postprandial myofibrillar protein degradation.

Dietary protein and absorbed amino acids are other factors that regulate postprandial muscle protein degradation. Goodman and del Pilar Gomez have reported that myofibrillar protein degradation was decreased by refeeding with a diet including protein or amino acids. We therefore examined the effect of dietary protein on the muscle protein degradation after food intake. When rats were fed on a complete diet, the rate of myofibrillar protein degradation decreased at 3 and 6 h after refeeding. In contrast, when the rats were fed on a protein-free diet, the rate of myofibrillar protein degradation was unchanged from the pre-feeding level. Furthermore, the inhibitory effect of dietary protein on myofibrillar protein degradation tended to be stronger when the rats were fed on a 20% or 40% protein diet than when fed on a 10% protein or protein-free diet (Fig. 5). These results strongly indicate that dietary protein participated in the inhibition of muscle protein degradation after food intake. Louard et al. have demonstrated that an infusion of branched-chain amino acids or leucine suppressed whole-body and skeletal muscle proteolysis. Therefore, a possible mechanism for the reduced protein degradation after food intake is one mediated by branched-chain amino acids, particularly leucine from dietary proteins, and these amino acids may directly or indirectly affect proteolytic systems such as the lysosome (autophagosome) and/or ATP-ubiquitin-dependent proteolytic system. Muscle protein turnover after food intake may be influenced by many other factors such as dietary energy, circulating T₃, and IGF-1. Since dietary protein may act with other factors to inhibit muscle protein degradation, it is necessary to confirm the effects of these factors on the postprandial regulation of muscle protein degradation by further experiments.

In conclusion, we have demonstrated that dietary protein suppressed the postprandial muscle protein degradation. Thus, an accumulation of muscle protein coupled with an increase in protein synthesis and a decrease in protein degradation was regulated immediately after food intake.

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