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Supplementation of protein-free diet with whey protein hydrolysates prevents skeletal muscle mass loss in rats

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ABSTRACT

Muscle mass loss is induced by aging, several catabolic diseases, and malnutrition. It is well known that ingestion of whey protein and its hydrolysates (WPH) is effective in stimulating muscle protein synthesis. However, these studies focused on the acute up-regulation of muscle protein synthesis, and few studies have investigated the effect of whey protein and WPH on muscle mass during chronic malnutrition. The aim of the present study was to investigate the effect of 7 days supplementation of whey protein and WPH on muscle reduction in Wistar rats fed a protein-free (PF) diet. Wistar rats were fed either a standard diet (containing 20% protein) or a PF diet during the experimental period. Those fed a PF diet received a dietary supplement containing an amino acid mixture, whey protein, or WPH for 7 days. The weight of the extensor digitorum longus decreased in rats fed a PF diet supplemented with the amino acid mixture or the whey protein. However, this decrease was partially but significantly suppressed in the group fed the WPH supplement. Additionally, administration of WPH induced a postprandial increase in plasma essential amino acids, branched-chain amino acids, and leucine concentration compared with animals fed the amino acid mixture or the whey protein. These results suggest that 7 days supplementation of the diet with WPH suppressed muscle weight loss in rats fed a PF diet.

1. Introduction

Skeletal muscle mass is regulated by the balance between the rates of muscle protein synthesis and degradation [1]. A reduction of muscle mass occurs when muscle protein degradation exceeds protein synthesis. This muscle turnover balance is affected positively by exercise and dietary protein and negatively by aging, several catabolic diseases, and malnutrition [1–3]. Reduction in muscle mass, which results in decreased mobility, impairs daily activity. Muscle wasting with aging, known as sarcopenia, induces the loss of functional immobility and independence [3]. Therefore, sarcopenia is becoming an issue of public concern. Positive muscle protein balance is essential for the prevention of muscle decline and the maintenance of muscle mass.

Dietary protein is an important nutrient for muscle metabolism because it increases the rate of protein synthesis and decreases that of protein degradation [4,5]. Ingestion of protein increases plasma amino acids, and the increased plasma amino acids are used as precursors for protein synthesis. Furthermore, essential amino acids (EAA), especially branched chain amino acids (BCAA), have a particular role in the regulation of muscle protein synthesis [6,7]. Many studies have demonstrated that BCAA stimulate muscle protein synthesis and facilitate the initiation of mRNA translation through the mammalian target rapamycin signaling pathway [8–11]. Besides stimulating protein synthesis, BCAA (leucine) supplementation is thought to suppress protein degradation by inhibiting atrogin-1 expression and autophagy activity [12,13]. Recently, Sugawara et al. reported a reduction of muscle mass is suppressed by leucine supplementation in rats fed a protein-free (PF) diet [14,15]. Consumption of amino acids has a beneficial effect on muscle mass; however, there is a practical problem with the cost of supplementation.

Milk protein—especially whey protein—is a rich source of BCAA

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and is thought to have beneficial effects on skeletal muscle turnover. Ingestion of whey protein strongly stimulates muscle protein synthesis in a dose-dependent manner [16,17]. This property is attributed to the faster digestion and absorption kinetics of whey protein, which result in a greater postprandial increase in plasma amino acids and further stimulate skeletal muscle synthesis [18,19]. Furthermore, several studies have investigated the effect of the ingestion of different types of whey protein on muscle synthesis. Morifuji et al. demonstrated that consumption of whey protein hydrolysates (WPH) causes a greater increase in the plasma amino acids concentration than the ingestion of intact whey protein [20]. Kanda et al. reported that whey protein hydrolysates are superior to their constituent amino acids for stimulating muscle synthesis [21,22].

Although acute studies provide some evidence concerning the effects of intact whey protein and WPH on muscle protein synthesis [16,21,22], there are few studies that assess which forms of whey protein are the most effective in preventing muscle mass loss during chronic muscle protein degradation. In this study, we used PF-fed rats as a model of muscle reduction model to investigate the effect of WPH or intact whey protein on chronically decreased muscle mass. We tested whether 7days WPH administration prevented PF diet-induced muscle degradation.

2. Materials & methods

2.1. Animals

Nine-week-old male Wistar rats were purchased from SLC (Shizuoka, Japan). The rats were housed under a 12 h light and 12 h dark cycle (light 8:00 a.m.–8:00 p.m.). They were individually housed in polycarbonate cages and were maintained at 24 ± 1 °C and 55 ± 5% relative humidity. They were allowed free access to water and a 20% casein (20% Cs) diet according to AIN-93G (Table 1). The use of animals in this study was in accordance with a protocol that was approved by the Morinaga Milk Industry CO., Ltd. Animal Care Committee.

2.2. Experimental protocol

After 1 week of receiving a 20% Cs diet for acclimatization, the rats were randomly assigned to the following groups:

1) 20% casein diet (20% Cs)
2) PF diet supplemented with whey protein amino acids (WAM)
3) PF diet supplemented with whey protein concentrate (WPC)
4) PF diet supplemented with WPH (WPH)

Table 1
Composition of the experimental diets used in this study.

Component	Composition (g/100g)	
	20% Cs	PF
α Corn Starch	13.2	22
B Corn Starch	39.75	51.25
Casein	20	0
Sucrose	10	10
Soybean Oil	7	7
Cellulose	5	5
AIN93G Mineral Mix	3.5	3.5
AIN93G Vitamin Mix	1	1
L-Cystine	0.3	0
Choline Bitartrate	0.25	0.25
Total	100	100

The 20% Cs group was fed a 20% casein diet, and the other groups were given a protein-free diet ad libitum throughout the study. Table 1 shows the composition of the experimental diet. A suspension containing the appropriate test sample (2.6 mL) was orally administered once a day, every day, for 7 consecutive days. Test solutions contained WAM, WPC (protein 77.04%, fat 5.05%, carbohydrate 9.47%, ash 2.57%, moisture 5.89%; Millei Co., Ltd), or WPH (protein 79.2%, fat 0.3%, carbohydrate 11.1%, ash 4.8%, moisture 4.6%; Morinaga Milk Industry Co., Ltd) dissolved in distilled water. The protein content of the test solutions was 200 mg/ml. The amino acid composition of WAM was equivalent to that of WPC (Table 2), and each amino acid was prepared using material purchased from Kyowa Hakko Bio Co., Ltd. or SIGMA. WPH was enzymatically obtained from whey protein concentrate. Its degree of hydrolysis was 22.8% and average molecular weight was 440 Da. As the control, the 20% Cs group was administered the same volume of distilled water. During this period, food intake was monitored every day, and body weight was measured on Day 1 and Day 7. On the evening of Day 7, the 20% Cs and PF diets were removed, and the rats were fasted for at least 16 h. The next day, the rats were orally administered 2.6 mL of test solution, and blood samples were drawn from the caudal vein 0, 5, 15, 30, 60 min after the consumption of the test solutions. Blood was collected into heparin–EDTA-containing tubes and was immediately centrifuged at 1000 g at 4 °C for 10 min. Plasma samples were frozen in liquid nitrogen and stored at −80 °C for further analysis. Then, the rats were deeply anesthetized with isoflurane, and the extensor digitorum longus (EDL) and soleus muscles were removed and their masses measured.

2.3. Plasma free amino acids analysis

Plasma free amino acid levels was measured using a Hitachi amino acid analyzer, model L-8900 (Hitachi High-Tech Science Co., Ltd.). Trichloroacetate (final 5%) was added to the plasma samples, and then they were centrifuged at 12,000 g for 20 min at 4 °C. Plasma free amino acids were determined by subjecting the supernatant to high-performance liquid chromatography.

2.4. Statistical analysis

The results are presented as means + SEM. The statistical analyses were performed using PASW Statistics for Windows version 17 (SPSS Japan). Comparison of data among the PF-fed rats was conducted using one-way ANOVA, and when a significant

Table 2
Composition of the whey amino acid mixture (WAM), WPC and WPH.

Amino acid	WAM & WPC (%)	WPH (%)
Ala	5.03	5.48
Arg	2.42	2.43
Asx	10.30	10.78
Cys	2.48	1.61
Glx	17.35	18.71
Gly	1.71	1.82
His	1.68	1.83
Ile	6.42	6.21
Leu	10.80	10.07
Lys	8.94	9.01
Met	1.96	1.40
Phe	3.16	3.07
Pro	5.84	5.98
Ser	4.86	4.94
Thr	6.68	7.32
Thy	3.02	2.95
Trp	1.62	1.23
Val	5.75	5.16