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Original article

A soy, whey and caseinate blend extends postprandial skeletal muscle protein synthesis in rats

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SUMMARY

Background & aims: Blends of dairy and soy protein are used in commercial sports nutrition products; however, no studies have systematically compared blends to isolated protein sources and their effects on muscle protein synthesis (MPS). Dairy whey protein (WP), soy protein isolate (SP), and two blends (Blend 1 and Blend 2) consisting of ratios of 50:25:25 and 25:50:25 for whey:caseinate:soy, respectively, were evaluated for their ability to affect MPS.

Methods: Male Sprague–Dawley rats were trained to eat 3 meals/day: a 4 g meal at 0700–0720 hours followed by *ad lib* feeding at 1300–1400 hours and 1800–1900 hours. After ~5 days of training, fasted rats were administered their respective 4 g meal at 0700–0720 hours and an intravenous flooding dose of $^{2}H_{5}$-phenylalanine 10 min prior to euthanasia. Individual rats were euthanized at designated postprandial time points. Blood and gastrocnemius samples were collected and the latter was used to measure mixed muscle protein fractional synthetic rates (FSR).

Results: Plasma leucine concentrations peaked in all groups at 90 min and were still above baseline at 300 min post-meal. FSR tended to increase in all groups post-meal but initial peaks of FSR were different times (45, 90 and 135 min for WP or SP, Blend 1 and Blend 2, respectively). Blend 2 had a significantly higher FSR compared to WP alone at 135 min ($P < 0.05$).

Conclusions: Single source proteins and protein blends all enhance skeletal MPS after a meal, however, Blend 2 had a delayed FSR peak which was significantly higher than whey protein at 135 min.

1. Introduction

The ingestion of essential amino acids and/or high-quality proteins after resistance exercise enhances the rate of muscle protein synthesis (MPS) as compared to exercise alone.[1–8] In a 2009 position paper the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine concluded that "intact high-quality proteins such as whey, casein, or soy are effectively used for the maintenance, repair, and synthesis of skeletal muscle proteins in response to training".[9] Most recently, research has focused on the leucine content of the above mentioned proteins due to the central role of this amino acid in inducing MPS through the mammalian target of rapamycin (mTOR) pathway in both human and rodent skeletal muscle.[10–15] A majority of studies looking at MPS have utilized amino acid mixtures or whey protein isolates and have been typically designed to examine protein accretion during the first 1–4 h post-ingestion. Researchers have focused on the 1–4 h post-ingestion time period since muscle protein synthesis is optimally stimulated by whey or amino acid mixtures during this interval due to the rapid digestion and absorption rates of amino acids from these sources.[16–18] On the other hand, proteins such as casein and soy have slower digestion rates compared to whey protein and this may affect time course for stimulating MPS.[18] For example, Reitelseder et al.[19] found in humans that if the sampling time was extended to 6 h after a single bout of exercise (i.e., 3 h longer than most study protocols employing whey protein), casein elicited a similar muscle protein anabolic response as compared to whey.

Acute muscle synthesis research has focused on purified essential amino acid mixtures or isolated protein; however it has recently been suggested that ingestion of protein blends following exercise may be more beneficial for muscle recovery.[20] If a sports

Abbreviations: MPS, Muscle protein synthesis; WP, Whey protein isolate; SP, Soy protein isolate; FSR, Fractional synthetic rate.

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Table 1
Composition of experimental diets.

Ingredient	Diets (g/1000 g diet)			
	100% Whey	100% Soy	Blend 1	Blend 2
Soy protein isolate[a]	0	241	59	59
Milk whey protein isolate[b]	234	0	118	59
Caseinate, sodium[c]	0	0	59	118
DL-Methionine	3	3	3	3
Corn starch	290	290	290	290
Maltodextrin 10	136	136	136	136
Sucrose	101.5	101.5	101.5	101.5
Cellulose, BW200	53.7	53.7	53.7	53.7
Cocoa butter	35.2	35.2	35.2	35.2
Linseed oil	4.2	4.2	4.2	4.2
Palm oil	49.3	49.3	49.3	49.3
Safflower oil	26.8	26.8	26.8	26.8
Sunflower oil, Trisun extra	25.4	25.4	25.4	25.4
Salts, S10026	10	10	10	10
Dicalcium phosphate	13	13	13	13
Calcium carbonate	5.5	5.5	5.5	5.5
Potassium citrate, 1 H20	16.5	16.5	16.5	16.5
Vitamins, V13401	10	10	10	10
Choline bitartrate	2	2	2	2
TBHQ	0.03	0.03	0.03	0.03
Protein, kcal%	20	20	20	20
Carbohydrate, kcal%	50	50	50	50
Fat, kcal%	30	30	30	30

[a] SUPRO®XF8021, Solae, LLC.
[b] Provon® 190, Glanbia Nutritionals, Inc.
[c] Sodium Caseinate Ultra Supreme, Erie Foods International, Inc.

nutrition beverage included a blend of slow, medium, and quickly digested proteins, the nutrient-induced enhancement of MPS may be prolonged during post-exercise recovery. Another potential advantage of consuming a protein blend is that each of the high-quality proteins have different amino acid compositions[21,22] and a blend of proteins would be expected to provide a higher average concentration of a wider variety of amino acids, some of which have unique and important functions independent of their role as substrates for protein synthesis. For example, whey has a higher relative content of leucine than soy or casein, but soy is relatively rich in glutamine and arginine both of which may also play an important role in up regulating MPS.[20]

In the current study dairy whey protein isolate (WP) and soy protein isolate (SP) alone as well as these proteins combined in two different blend formulations were evaluated for their ability to affect MPS. The blends were prepared as follows: Blend 1 and Blend 2 consisted of ratios of 50:25:25 and 25:50:25 of whey protein isolate:caseinate:soy protein isolate, respectively. The hypothesis tested was that the protein blends would stimulate MPS over a longer period of time postprandially compared to the isolated protein sources, soy or whey. The study used a commonly used rat model to assess muscle protein synthesis. No exercise was used in this model so that the effects of diet alone on stimulating muscle protein synthesis could be assessed.

2. Methods

2.1. Animals and diets

Male Sprague–Dawley rats weighing approximately 300 ± 15 g from Harlan Teklad were maintained in a temperature controlled room, with a 12-h light/dark cycle and allowed free access to food and water during acclimation. Purified diets were prepared by Research Diets, Inc., New Brunswick, NJ USA and provided 20%, 50% and 30% of energy from protein, carbohydrates and fat (Table 1). The study protocol was reviewed and approved by the Institutional Animals Care and Use Committee and conducted by Seventh Wave Laboratories, at the Department of Comparative Medicine, St. Louis University. The animals were cared for according to the NIH Guide for the Care and Use of Laboratory Animals.

The experimental protocol was adapted from a previously reported method[13] and animals in the current study were matched for age, weight and diet macronutrients. Animals were trained to meal feed 3 meals a day; a morning meal (4 g) presented at 0700 hours for approximately 20 min; an *ad libitum* meal for 1 h at 1300 hours; and an *ad libitum* meal for 1 h at 1800 hours for 5 days using the respective treatment diets for each group. Food consumption was not monitored during training but diet consumption was accurately recorded for animals after presentation of the 4 g test meal. On day 6 animals were feed-deprived for 12 h and then were fed the 4 g meal at 0700 hours for approximately 20 min. Animals ($n = 6$/group/time point) were then killed at the following time points: Time 0, 45, 90, 135, 180 and 300 min after the 4 g meal. Animals were anesthetized by CO_2 and killed by exsanguination. Blood was taken by cardiac puncture and plasma collected. Left and right gastrocnemius muscles were excised, rinsed with PBS, and immediately frozen in liquid nitrogen.

2.2. Determination of muscle protein synthesis

MPS was measured in skeletal muscle (gastrocnemius) using the flooding dose method as previously described by Norten et al.[13] Ten minutes prior to sacrifice animals were administered a 40% enriched L-[$^2H_5$] phenylalanine solution (150 mmol/L; Cambridge Isotopes) through the tail vein (1 ml/100 g) at 150 μmol/100 g. Gastrocnemius muscle was collected 10 min following tracer injection and was immediately cooled in liquid nitrogen and stored at −80 °C. Muscle tissue samples were ground, and intracellular free amino acids and muscle proteins were extracted as previously described.[23] Muscle intracellular free enrichment of phenylalanine was determined by gas chromatography-mass spectrometry (GCMS, 6890 Plus GC, 5973N MSD, 7683 autosampler, Agilent Technologies, Palo Alto, CA).[23] Mixed muscle protein-bound phenylalanine enrichment was analyzed by GCMS after protein hydrolysis and amino acid extraction[23] using the external standard curve approach.[24] We calculated the fractional synthetic rate (FSR) of mixed muscle proteins by measuring the incorporation rate of the phenylalanine tracer into the proteins using the precursor-product model to calculate the synthesis rate:

$$\mathrm{FSR} - (\Delta E_b \times 100)/[E_{ic} \times t]$$

where ΔE_b is the protein-bound labeled phenylalanine enrichment, E_{ic} is the phenylalanine enrichment in the free intracellular pool, and t is the time between the labeled phenylalanine injection and the collection of muscle tissue. Data are expressed as percent per day.

FSR was measured following the 0700 hours meal in muscles taken from an N of six rats for each time point. One blood sample per euthanisation was obtained at each time point. An area under the curve (AUC) was calculated as an overall FSR for each diet treatment to obtain an estimate of the relative abilities of the proteins to stimulate MPS over time.

2.3. Plasma amino acid concentrations

Blood was centrifuged at 1800 × *g* for 10 min at 4 °C. Plasma amino acids were analyzed at ABC Laboratories (Columbia, MO) by HPLC using the AccQ-Tag Kit (Waters; Milford, MA).

2.4. Intramuscular branched chain amino acid concentrations

Concentrations of leucine, isoleucine and valine and were determined in muscle intracellular fluid using appropriate internal standards as previously described by Wolfe et al.[23]