Nutrition 30 (2014) 876–881

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Whey protein modifies gene expression related to protein metabolism affecting muscle weight in resistance-exercised rats

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ARTICLE INFO

Article history:
Received 28 August 2013
Accepted 5 December 2013

Keywords:
Exercise
Dietary protein
Protein synthesis
Protein degradation
mTOR
MAFbx
MuRF-1

ABSTRACT

Objective: The aim of this study was to evaluate the effects of resistance exercise on the mRNA expression of muscle mammalian target of rapamycin (*mTOR*), muscle-specific RING finger-1 (*MuRF-1*), and muscle atrophy F-box (*MAFbx*) in the presence or absence of whey protein ingestion. We hypothesized that resistance exercise in combination with whey protein ingestion alters the gene expression of proteins related to muscle protein synthesis (*mTOR*) and/or degradation (*MuRF-1* and *MAFbx*), thus affecting muscle weight gain in rats.

Methods: Thirty-two male Fischer rats were randomly assigned to the following four experimental groups (n = 8/group): Control sedentary, control exercised, whey protein sedentary, and whey protein exercised. Exercise consisted of inducing the animals to perform sets of jumps for 8 wk. Body weight gain, muscle weights, food intake, and feeding efficiency were evaluated. Gene expressions were analyzed by quantitative real-time reverse transcription polymerase chain reaction. Statistical evaluation was performed using a two-way analysis of variance with a Tukey post hoc test.

Results: Whey protein exercised rats exhibited higher body and muscle weight gain compared with control-exercised rats ($P = 0.032$). The expression of *mTOR* was reduced by exercise but increased when whey protein was consumed as a dietary protein ($P = 0.005$). *MuRF-1* expression was reduced by exercise ($P < 0.001$), whereas *MAFbx* was reduced only by whey protein ingestion ($P = 0.008$) independent of exercise.

Conclusions: A reduction in *MAFbx* gene transcription induced by whey protein and the interaction between exercise and whey protein ingestion on *mTOR* gene expression contributed significantly to differences in body and muscle weight gain.

Introduction

Regular resistance exercise (RE), such as weightlifting, in combination with adequate protein consumption, efficiently stimulates muscle growth, which results from a cumulative increase in muscle protein synthesis, a decrease in muscle protein degradation, or a combination of the two. The rate of protein synthesis is mediated by the activation of a cellular network of

This study was supported by the Federal University of Ouro Preto (UFOP, Minas Gerais, Brazil), the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG, Minas Gerais Research Support Foundation), and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, National Council for Scientific and Technological Development).

FKH was responsible for generation, collection, assembly, analysis, and interpretation of the data; drafting of the manuscript. CLdeBM was responsible for analysis and interpretation of the data. LXN was responsible for generation, collection, assembly, and analysis of the data. RCdosS was responsible for drafting and revision of the manuscript. MLP was responsible for study conception and design; data interpretation; and manuscript revision. MES was responsible for study conception and design; generation, collection, assembly, analysis, and interpretation of the data; and manuscript revision. All authors approved the final version of the manuscript.

The authors report no conflicts of interest.

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http://dx.doi.org/10.1016/j.nut.2013.12.007

signaling pathways involving the mammalian target of rapamycin (mTOR), a central serine/threonine kinase that integrates several different upstream and downstream signals that regulate mRNA translation [1,2]. Muscle protein degradation is mediated primarily (80%–90%) by the ubiquitin proteasome system (UPS) [3], which includes the following three components that participate in ubiquitin transfer reactions: Ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligases (E3). E3 ligases, particularly muscle atrophy F-box (MAFbx) and muscle-specific RING finger-1 (MuRF-1), are key enzymes mediating muscle protein loss and are overexpressed in numerous catabolic conditions [4,5].

The phosphorylation of mTOR can be enhanced by amino acids and proteins, especially those with high leucine content [2,6]. Therefore, whey protein (WP) deserves special attention because it is present in a variety of sports supplements, and several benefits of WP for athletes have been reported [7,8]. WP induces mTOR phosphorylation to a greater extent than other protein sources, such as soy, in treadmill-exercised rats [9] and prolongs mTOR signaling in response to RE in humans [10]. However, the effects of RE in combination with WP ingestion on *mTOR* gene expression are not well known.

Conflicting data have been reported on the effects of RE on the E3 ligases, *MAFbx*, and *MuRF-1*. Overexpression of E3 ligases mRNA was observed after a single RE session [11,12], whereas other studies reported different effects of chronic RE on *MuRF-1* and *MAFbx* mRNA expression [3,13]. Additionally, the effects of combined RE and WP ingestion on *MAFbx* and *MuRF-1* mRNA expression are less clear. We hypothesize that RE in combination with WP ingestion might alter the gene expression of proteins related to muscle protein synthesis and/or degradation in rats and thus affect muscle weight gain.

Therefore, this study aimed to evaluate the mRNA expression of *mTOR*, *MAFbx*, and *MuRF-1* in rats subjected to a RE protocol for 8 wk. Additionally, we evaluated the effects of WP on RE-induced gene expression and how these adaptations affect body and muscle weight gain.

Materials and methods

Animals and groups

Thirty-two male Fischer rats (60 d old) weighing approximately 110 g were used in the experiment. The animals were housed individually in galvanized wire metabolic cages in a room with controlled temperature (23 ± 1°C), relative humidity (55 ± 10%), and a 12-h light/dark cycle. The animals received care in accordance with the guidelines of the Canadian Council on Animal Care. Rats were randomly distributed into four experimental groups (n = 8/group) as follows: Control sedentary (CS) control exercised (CE) whey protein sedentary (WS) and whey protein exercised (WE).This research was approved by the Ethical Committee of Federal University of Ouro Preto–protocol no. 036/2008.

Diets

The CS and CE rats were fed the AIN-93 M standard diet [14], and the WS and WE animals received the AIN-93 M diet modified with WP instead of control protein. The compositions of the diets are presented in Table 1, and the amino acid compositions of the proteins (casein as a control and WP) are presented in Table 2. Food and water were provided ad libitum. Body weight and food consumption were measured weekly. Food consumption was corrected for losses from spilling.

Exercise training program and experimental procedures

Exercised rats were submitted to a RE program for 8 wk, as previously reported [15], to mimic a weightlifting-training model for hind limb muscles.

During an adaptation period, rats from each exercised group (CE and WE) were subjected to swimming without weights for 15 min in a 40-cm deep swimming pool with a water temperature of 32 ± 1°C for 1 wk. After the adaptation period, the exercise training consisted of inducing the animals to perform jumps in a circular plastic container with a water level corresponding to 150% of their body length. Weights were attached to the animal's chest to promote submersion. When the rats touched the bottom of the container, they had to jump to emerge from the water to breathe. Water in the container and the weights attached to the animal's chest generated resistance during the exercise. The animals performed four sets of 10 jumps per day, five times per week for 8 wk. A 1-min resting interval was included between each set of jumps. The exercise intensity was increased weekly by changing the weights according to the animal's body weight (25% of body weight in week 1, 30% in week 2, 35% in week 3, 40% in week 5, 50% in week 6, and 55% in weeks 7 and 8). During the RE sets, sedentary animals were kept in a similar aquatic environment consisting of a 5-cm deep swimming pool. At the end of the eighth training week, and within 24 h after the last training session, all rats were fasted for 12 h, anesthetized by isoflurane inhalation (4%), and sacrificed by cardiac puncture. The gastrocnemius and extensor digitorum longus (EDL) muscles were immediately excised, washed, weighed, and stored at −80°C until further analysis. All rats were evaluated for body weight gain, muscle weights, food intake, and feeding efficiency. For quantitative real-time reverse transcription polymerase chain reaction (PCR), six animals per group were randomly selected. However, for MuRF-1 mRNA expression, two rats from the WS group (WS n = 4) and one from the WE group (WE n = 5) were excluded because no amplification during real-time PCR was observed.

Table 1
Compositions of the experimental diets

Ingredients (g/1000 g)	CS/CE	WS/WE
Casein*	140	–
Whey protein†	–	150
Mineral mixture‡	35	35
Vitamin mixture§	10	10
Soybean oil	40	40
Sucrose	100	100
Cellulose	50	50
Choline	2.5	2.5
Cornstarch	622.5	612.5
Total	1.000	1.000

CE, control exercised; CS, control sedentary; WE, whey protein exercised; WS, whey protein sedentary

* Isofar (Rio de Janeiro, Brazil), containing 85% protein as determined by the Kjeldahl method.

† Probiótica, (São Paulo, Brazil), containing 80% protein as determined by the Kjeldahl method.

‡ Mineral mixture for the AIN-93M diet [14].

§ Vitamin mixture for the AIN-93M diet [14].

Total RNA isolation and cDNA synthesis

Total RNA was isolated from 40 to 50 mg of freeze-dried gastrocnemius muscle using the guanidine thiocyanate method with a lysis buffer solution

Table 2
Amino acid composition of casein (control) and whey protein used as a dietary protein source, and the minimum requirements for rodent diets (g/100 g protein)

Amino acid	Casein	Whey protein	Minimum requirements*
Threonine	3.7	8.0	3.7
Valine	5.5	6.3	5.6
Isoleucine	4.2	7.2	4.7
Leucine	8.4	11.2	8.7
Lysine	6.9	8.8	7.3
Methionine	2.6	2.2	2.6
Phenylalanine	4.3	3.1	4.9
Tryptophan	1.2	1.9	1.3
Tyrosine	4.8	3.2	–
Cysteine	0.4	2.7	–
Aspartic acid†	6.3	11.9	–
Arginine	2.9	2.2	–
Serine	4.9	5.3	–
Histidine	2.2	2.1	–
Glutamic acid‡	18.8	18.9	–
Glycine	1.7	1.7	–
Alanine	2.7	5.3	–
Proline	10.6	7.8	–

* Estimated minimal nutrient composition of the AIN-93M rodent diets [14].

† Aspartic acid: aspartic acid + asparagine.

‡ Glutamic acid: glutamic acid + glutamine.