Clinical Nutrition xxx (2016) 1–9

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Randomized control trials

Anabolic effects of leucine-rich whey protein, carbohydrate, and soy protein with and without β-hydroxy-β-methylbutyrate (HMB) during fasting-induced catabolism: A human randomized crossover trial

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ARTICLE INFO

Article history:
Received 11 March 2016
Accepted 6 May 2016

Keywords:
Whey
Soy
Protein
Nutrition
Fasting
Leucine
HMB

SUMMARY

Background: Protein-rich beverages are widely used clinically to preserve muscle protein and improve physical performance. Beverages with high contents of leucine or its keto-metabolite β-hydroxy-β-methylbutyrate (HMB) are especially anabolic in muscle, but it is uncertain whether this also applies to catabolic conditions such as fasting and whether common or separate intracellular signaling cascades are involved.

Objective: To compare a specific leucine-rich whey protein beverage (LWH) with isocaloric carbohydrate-(CHO), soy protein (SOY), and soy protein +3 g HMB (HMB) during fasting-induced catabolic conditions.

Design: Eight healthy lean male subjects underwent four interventions (LWH, CHO, SOY, and HMB) using a randomized crossover design. Each trial included a 36 h fast and consisted of a 3 h basal fasting period and a 4 h 'sipping' period.

Results: Forearm net balances of phenylalanine (NB_{phe}, measure of net protein loss) improved for all groups ($p < 0.05$), but more prominently so for LWH and HMB compared with SOY ($p < 0.05$). Muscle protein phosphorylation of mammalian target of rapamycin (mTOR) and its downstream targets eukaryotic translation factor 4E-binding protein 1 (4EBP1) and ribosomal S6 kinase 1 (S6) were distinctly increased during LWH consumption ($p < 0.05$). The ratio between autophagy protein microtubule-associated protein 1 light chain-3β II and I (LC3II/LC3I, a measure of autophagy activity) was decreased during LWH and SOY intake compared with the fasting period ($p < 0.05$).

Conclusion: LWH and HMB have superior anabolic effects on muscle protein kinetics after 36 h of fasting, and LWH distinctly activates the mTOR pathway. These novel findings suggest that leucine-rich whey protein and/or HMB are specifically beneficial during fasting-induced catabolic conditions.

1. Introduction

Protein supplementation and physical exercise are both known to have anabolic effects in muscle and are therefore widely used by athletes and others to improve physical performance. Clinically, protein supplementation is used to prevent whole body and muscle protein loss due to catabolic illness [1], immobilization, and malnutrition [2]. It is however not well established how protein supplementation affects muscle tissue protein metabolism during catabolic conditions.

Under catabolic conditions such as fasting the human body primarily utilizes fat and protein compared to the fed state, which is characterized by high glucose utilization [3–5]. Proteins are

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http://dx.doi.org/10.1016/j.clnu.2016.05.004
0261-5614/

Abbreviations

4EBP1	Eukaryotic translation factor 4E-binding protein 1
AS160	Akt substrate of 160 kDa
BHB	β-hydroxybutyrate
BMI	Body mass index
CHO	Carbohydrate group
EE	Energy expenditure
FBX32	F-Box protein 32
FFA	Free fatty acids
FoxO3a	Forkhead transcription factor
HMB	β-hydroxy-β-methylbutyrate
LC3β	Autophagy protein microtubule-associated protein 1 light chain-3β
LWH	Leucine-rich whey protein group
mTOR	Mammalian target of rapamycin
MURF1	Muscle RING-finger protein-1
NB_{phe}	Net balance of phenylalanine
P62	Nucleoporin P62
Ra_{phe}	Rate of appearance of phenylalanine
Rd_{phe}	Rate of disappearance of phenylalanine
S6	Ribosomal S6 kinase 1
SOY	Soy protein group
ULK1	Unc-51 like autophagy activating kinase 1

primarily stored as muscle tissue and are broken down and released during prolonged fasting where no exogenous supply of amino acids is provided [3]. Whey protein supplementations rich in the amino acid leucine have demonstrated muscle anabolic effects compared to other protein sources [6–8], but these studies investigated healthy subjects without any metabolic stress. Leucine is transaminated to α-ketoisocaproate (KIC), which is mainly oxidized to isovaleryl CoA, but a minor part is oxidized to the keto-metabolite β-hydroxy-β-methylbutyrate (HMB) [9]. HMB supplementation also has muscle anabolic effects [10,11], but again these studies have been conducted in healthy subjects devoid of any catabolic stress. The anabolic effects of leucine-rich protein supplements could to some extent be explained by the higher HMB concentrations following leucine consumption [9].

Insulin, leucine, and HMB all exert specific anabolic effects in muscle tissue and appear to activate independent intracellular signaling pathways, which converge and eventually activate the mammalian target of rapamycin (mTOR). Signaling downstream of mTOR include 4E-binding protein 1 (4EBP1) and the ribosomal S6 kinase 1 (S6) [8,12–15].

The beneficial effects of leucine have been shown to be independent of age and route of administration (enteral compared with parenteral) [16–20]. However, very little is known about the effects of leucine-rich protein supplementation to humans during catabolic conditions such as fasting. Only few human studies have investigated the effect of leucine-rich protein supplementation after a longer period of fasting, and these studies have shown nitrogen-sparing effects [21–23]. These studies, however, did not include comparisons to other conventional nutrients and did not assess specific effects on muscle protein kinetics and signaling events. Whey protein has a high natural leucine content, but it is possible to isolate whey protein fractions with even higher leucine content.

Therefore, our study was designed to test i) whether a leucine-rich (=16% of total protein content) whey protein beverage is more anabolic in muscle under catabolic conditions compared with isocaloric carbohydrate, and isocaloric isonitrogenous soy protein with and without 3 g HMB enrichment and ii) whether leucine-rich whey protein specifically affects regulators of protein-synthesis and breakdown in muscle.

2. Subjects and methods

2.1. Subjects

Subjects were eligible for inclusion in the study if they were of male gender, had a body mass index (BMI) between 20 and 30 kg m^{-2}, were older than 20 years, and were healthy without regular intake of medication. All subjects were screened using a medical interview, physical examination, and a routine biochemistry test. Subjects were included in the study after oral and written informed consent had been obtained. The study day was postponed in the event of febrile illness during the preceding week. Each subject was instructed to avoid physical exercise 48 h before each trial and to eat in accordance with general nutritional recommendations (energy-distribution; 30% fat, 50–60% carbohydrates, and 10–20% protein) 24 h before fasting.

2.2. Ethics

The Central Denmark Region Ethics Committee approved the study in accordance with the Declaration of Helsinki [1-10-71-63-13] and the study was reported at www.clinicaltrials.gov (identification number NCT 01840098).

2.3. Study design and protocol

This study was designed as a randomized crossover-trial with four different study days. Each study day was separated with a minimum of 21 days. The primary investigator enrolled subjects and assigned them to the interventions using a computerized randomizer program. Subjects were blinded to the intervention. Furthermore, subjects were thoroughly instructed only to drink tap water ad libitum 36 h (total fasting with absolutely no calorie intake allowed) preceding each trial and arrived by car at the laboratory 07.00 AM. One arm was inserted with an intravenous catheter in a cubital vein and used for intravenous infusions. An additional intravenous catheter was inserted into a dorsal hand vein, and an electric warming cloth was wrapped around the hand to obtain arterialized blood [24]. A retrograde catheter was inserted into a deep cubital vein of the contralateral arm to collect venous blood samples.

All study days had an identical setup and did only differ as regards the content of nutrients in the beverage. The four interventions were:

i) A beverage containing carbohydrate **(CHO)**
ii) A beverage based on leucine-rich whey protein **(LWH)**
iii) A beverage based on soy protein **(SOY)**
iv) A beverage based on soy protein +3 g HMB **(HMB)**

2.4. Interventional beverages

The intervention-products were produced by Arla Foods Ingredients Group P/S (Viby, Denmark) and provided as powder

Please cite this article in press as: Rittig N, et al., Anabolic effects of leucine-rich whey protein, carbohydrate, and soy protein with and without β-hydroxy-β-methylbutyrate (HMB) during fasting-induced catabolism: A human randomized crossover trial, Clinical Nutrition (2016), http://dx.doi.org/10.1016/j.clnu.2016.05.004