EXERCISE METABOLISM RESEARCH GROUP
DEPARTMENT OF KINESIOLOGY, MCMASTER UNIVERSITY

PARTICIPANT INFORMATION SHEET AND CONSENT FORM

Supplementation of a Leucine-Enriched Dairy Protein Blend: Chronic and acute metabolic responses in young men and women

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Rational

The importance of protein in nutrition is momentous to one's health. Dietary proteins are essentially nutritional because of their constituent amino acids, which the body uses to synthesize its own proteins, as well as nitrogen-containing molecules that are essential for life (1). The Recommended Dietary Allowance (RDA) for daily protein intake in healthy adults is 0.8 $g \cdot kgBW^{-1} \cdot day^{-1}$ (2). Younger individuals who participate in recreational or competitive activities, however, often attempt to go beyond the RDA by using protein supplements to help increase performance. For example, Coburn et al. (2006) demonstrated that protein supplementation in conjunction with an eight-week resistance-training program elicited greater improvements in muscle hypertrophy and strength when compared to carbohydrate supplementation in untrained men. There were 3.2 to 17.5% increases in muscle cross-sectional area of the quadriceps femoris musculature and 14.5 to 30.3% strength gains in the leg extensors (3). Furthermore, several expert groups have recently advocated that certain individuals, such as older persons, should increase their daily intake range (1.0 to 1.5 $g \cdot kgBW^{-1} \cdot day^{-1}$) to support the preservation of muscle and function (4-6). Other conditions also place greater than normal demands on amino acids, such as hypercatabolic stressed states, like that experienced by burn patients who's muscle protein breakdown rate increases by impairment to inward transmembrane transport of circulating blood amino acids (7, 8). Deutz et al. (2011) found that an experimental enriched protein "medical food" comprised of 27% of calories as total protein resulted in postprandial increases in MPS rates for 5 hours by ~33% ($0.073 \pm 0.023\% \cdot h^{-1}$ to $0.097 \pm 0.033\% \cdot h^{-1}$) in advanced cancer patients. Thus, continually reassessing new and innovative nutrient therapies is necessary for extending the findings of existing studies after technological advances, and for providing nutritional support-to-support health. Therefore, we have developed a leucine-enriched protein bar that contains an effective dose of leucine in a high-quality protein matrix consisting of bovine milk proteins. Of all of the essential amino acids, leucine appears to be the most potent in activating anabolic signaling (16, 17, 19). The product weighs about 40g per serve, is low in energy (~180 kcal per serve), palatable, and does not interfere with appetite or subsequent meal intake.

It is well known that dietary protein is a powerful transient stimulator of the muscle protein synthetic rate (MPS) (9-11) whereby changes in MPS in response to feeding may be regulated by specific downstream target proteins of mammalian target of rapamycin signaling, such as S6K1, rpS6, and eIF2B (11). A meal deficient in protein, however, does not increase the rate of MPS because a rise in the bioavailability of amino acids does not occur (12, 13). In addition, the source of dietary proteins has been shown to impact postprandial blood levels of amino acids. The concept that certain types of proteins are "fast acting" or "slow acting" has been shown to affect the postprandial profile of amino acids appearing in the systemic circulation (13). Native whey and micellar casein are both dairy proteins that contain a similar amount of essential (EAA), but blood EAA levels increase faster and to a higher level after the consumption of whey protein (13, 14). Differences in gastric emptying, digestion and absorption kinetics between micellar casein and native whey are the underlying factors (13). Nonetheless, micellar casein protein has been shown to protract MPS in humans (15). Despite the significant amount of information gained with respect to both of these protein sources, the effects of combinatorial

formulations on the postprandial profile of amino acids appearing in the blood is less well known.

Purpose and hypothesis

The primary purpose of this study is to evaluate changes in integrated (day-to-day) rates of myofibrillar MPS in response to the product and a short-term resistance training program in young men and women. A secondary purpose is to assess the acute postprandial skeletal muscle protein signalling response to the ingestion of the product in young men and women. Based on the results of a previous diary protein bioavailability study (13) and our previous results (17, 18) we hypothesize that training + supplementation will result in greater integrated rate of muscle growth.

Experimental approach

In a cross-over, randomized controlled trial, 10 healthy young participants (5 men and 5 women) will be recruited to undergo exercise resistance training randomized to habitual diet or habitual diet and supplementation (2 x per training session). For two of the training sessions we will utilize a uni-lateral resistance exercise model to identify the acute effects of exercise and exercise + supplementation on the integrated rate of myofibrillar MPS within subject. This model enhances statistical power and eliminates between-subject differences impacting our outcomes. Throughout the study participants will record their macronutrient dietary intake. In addition, baseline body composition will be assessed with dual-energy x-ray absorptiometry.

Sample size estimate. We have previously detected significant alterations in myofibrillar fractional synthetic rates and lean muscle mass in response to step-reduction, and following the consumption of dietary protein and energy restriction (18-22). As a proof-of-concept investigation we will recruit *n=10* per group.

METHODS

Body composition will be determined using dual energy x-ray absorptiometry (DXA) scans. The DXA procedures use a small amount of radiation to determine the bodies fat, bone, and lean mass. The procedure takes approximately seven minutes and involves lying still on an open bed while the sensor passes over the body.

Integrated rates of protein synthesis will be assessed with the precursor-product method using ingested deuterium labeled water (70 %D_2O) as we have shown previously (23, 24). Briefly, to increase deuterium (^2H) enrichments in total body water to ~1%, participants will consume 4 D_2O doses (0.8ml/kg lean body mass per dose) on the loading day and 1 dose (0.8ml/kg lean body mass) on maintenance days. Total body water ^2H enrichment will serve as a surrogate for plasma alanine ^2H labeling (24-26). Daily saliva sampling will be continued until the end of the experimental trial to assess precursor pool enrichments. Muscle samples (~40–50mg) will be separated into myofibrillar and sarcoplasmic fractions and processed as previously described (27)

for analysis by gas chromatography combustion isotope ratio mass spectrometry (Metabolic Solutions, Nashua, NH). Briefly, muscle preparations will be analyzed for deuterated-alanine (^{2}H-alanine) with a Thermo Finnigan Delta V isotope ratio mass spectrometry coupled to a Thermo Trace GC Ultra with a gas chromatography combustion interface III and Conflow IV. Saliva samples will be analyzed for ^{2}H enrichment by cavity ring-down spectroscopy using a Liquid Water Isotope Analyzer with automated injection system.

Saliva Sample Instructions

A saliva sample will be taken every day during the study. The participant will use a labeled sampling tube corresponding to the specific day of the study and calendar date.

1. Saliva sample taken right before, or at least 30 minutes after, eating or drinking anything, including the heavy water (D2O). The best time to take it is first thing in the morning after waking up and before having breakfast.

2. Open the top lid of the plastic salivette vial by popping the lid off sideways.

3. Remove the white, cotton swab from inside the vial. Cotton swab is placed in the mouth.

4. Gently chew and suck on the cotton swab for about 60 seconds, until the swab is completely saturated with saliva (or until the participant can no longer prevent themself from swallowing the saliva produced).

5. Place the wet swab back inside the salivette vial and replace the cap, making sure it's securely on.

6. Do not spit into the plastic salivette vial at any time.

7. Place the salivette vial back inside the plastic Ziplock bag provided.

8. Place the Ziplock bag with the used salivette(s) inside a freezer.

9. Keep the Ziplock bag in a freezer until your next study appointment.

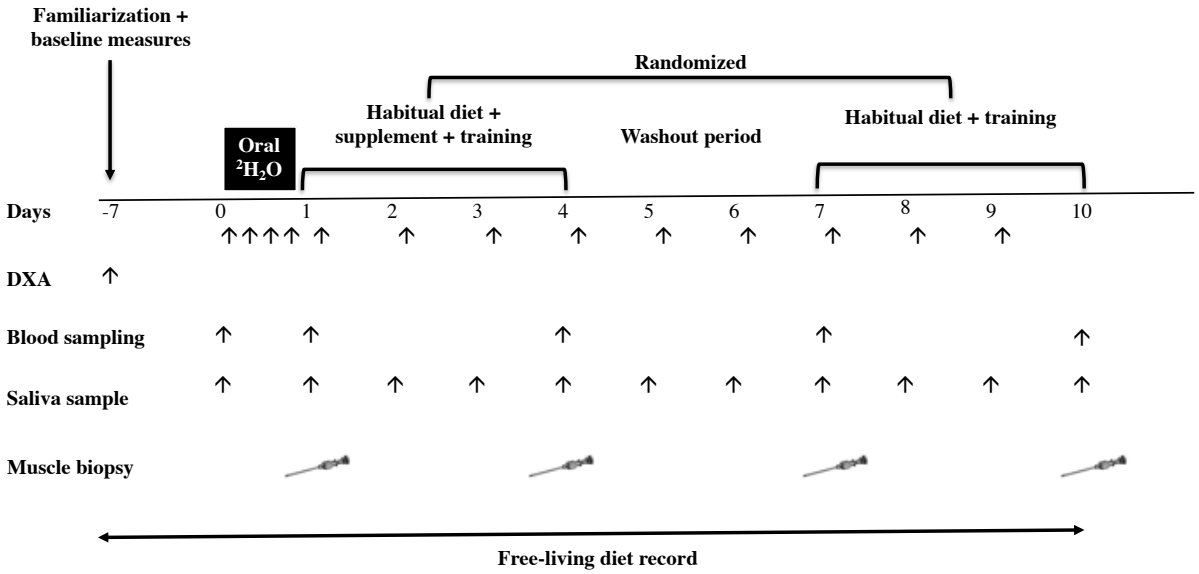
Please keep all unused salivettes stored at room temperature. Do not store unused salivettes in the freezer as these will be difficult to saturate. One Ziplock bag is for unused salivettes, the other Ziplock bag is for used salivettes (in the freezer).

Muscle biopsies will be sampled from the *vastus lateralis* using the Bergstrom technique before and after resistance training with either supplementation or habitual diet. Biopsies will be analysed for markers of protein synthesis utilizing Western blotting and PCR in addition to integrated MPS. The molecular mechanisms that underpin MPS in response to stimulation are complex, multifactorial and remain largely unknown. However, what is known is that proteins contained within the Akt-mTORC1-p70S6K1 signaling axis appear key. Thus, we propose to examine how the content of proteins contained within the Akt-mTORC1-p70S6K1 signaling axis change during our intervention. We also will examine changes in the DNA-protein ratio (an indicator of cell size) as well as mRNA expression of myogenin, MAFBx and MuRF1, some of which have been shown to change in response to muscle growth, using real-time PCR. ***Thus, our molecular analysis coupled with the use of the deuterated water methodology provides a mechanism-based clinical approach to study the impact of supplementation on skeletal muscle health.***

Venous Blood Sampling

A needle will be inserted into a forearm vein by a physician or a medically trained and certified member of the laboratory group, and a blood sample will be drawn (~5 mL or 1 teaspoons). The discomfort of this procedure is transient and is very similar to having an injection by a needle, e.g. when donating blood. Upon removal of the needle any discomfort should subside. During the course of the experiment, five blood draws will occur. In this experiment, the total blood taken is ~50 mL, which is 1/10th of the blood removed during a donation to a blood bank. This amount of blood loss is not enough to affect your physical performance in any way. After each blood sample has been taken pressure will be placed on the site in order to minimize bleeding and facilitate healing. The insertion of a needle for blood sampling is a common medical practice and involves few risks if proper precautions are taken. The needles are inserted under completely sterile conditions; however, there is a theoretical risk of infection. There is a chance of internal bleeding if adequate pressure is not maintained upon removal of the needle. This may cause some minor discomfort and could result in bruising/skin discoloration, which could last up to a few weeks. In very rare occasions, trauma to the vessel wall could result in the formation of a small blood clot, which could travel through the bloodstream and become lodged in a smaller vessel. However, we have never experienced such a complication after several thousand blood draws.

Resistance training will be composed of CrossFit style exercises individually customized to each participant (28). Prior to trial initiation, participants will report to the laboratory for familiarization of these types of exercises. In addition, for uni-lateral lower body resistance training sessions participants will perform 3 sets of leg press and leg extension exercises to volitional failure. 3-5 repetition maximum testing will be performed during the familiarization session to estimate 1-RM values for the leg press and leg extension exercises to allow for the determination of loads equal to approximately 80-90% 1-RM. For each exercise 6-10 repetitions/set will be completed.



Schematic overview of study design. DXA, dual-energy X-ray absorptiometry.

Statistics

Two-way repeated measures ANOVAs with time and treatment as factors will be used to compare differences between supplementation + exercise and habitual diet over the 8 days of resistance training. Where significant interaction is detected, post-hoc test will be applied to located these differences. Statistical significance is set at $P<0.05$. Calculations and analyses will be performed using the Prism V.7. and SPSS V.25. software packages.

Knowledge translation

The primary form of knowledge translation from these projects will be via conference presentations, published scientific papers, and press releases. We anticipate at least one major paper to arise from the project described here and at least that number of presentations at National and International meetings from Drs. Phillips and Traylor. We have a good publication record and would also opt for an online/open access journal or opt to pay an open access fee to allow wider use of our data. In addition, the PI gives at least 1-2 keynote lectures at various conferences both at National and International meetings. Hence, there will be relatively widespread dissemination of the data from these trials. As an adjunct member in the Department of Medicine, Faculty of Health Sciences, the PI is also positioned to present this work via Grand Rounds and other local seminars to clinicians to get information to those who can implement the findings into their practice. There will also be press releases that accompany each manuscript to more widely publicize the findings and garner the interests of clinically-oriented web services such as Web MD, Medscape, The Doctor's Page, and Healthcentral.com. We will also work with the McMaster Knowledge Translational Team, http://www.mcmaster.ca/cfh/knowledgetranslation.html - using McMaster PLUS, which is a premium 'literature service' at McMaster University designed to decrease the 'noise-to-signal

ratio' in articles from clinical journals. This service sends registered physicians e-mail updates with links to articles that are relevant to their discipline.

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