The effects of protein type and added leucine on post-exercise muscle protein synthesis

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Overall Objective: To define the properties of whey, casein, milk protein, as well as soy protein with and without additional leucine to augment post-exercise muscle protein synthesis when co-ingested with a carbohydrate containing recovery drink....

Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Protein and amino acid metabolism disorders NEC
Study type	Interventional

Summary

ID

NL-OMON42133

Source
ToetsingOnline

Brief title
Protein type and leucine on post-exercise protein synthesis

Condition

- Protein and amino acid metabolism disorders NEC
- Muscle disorders

Synonym
muscle anabolism; muscle protein deposition

Research involving
Human

Sponsors and support

Primary sponsor: Universiteit Maastricht
Source(s) of monetary or material Support: Ministerie van OC&W,Pepsico

Intervention

Keyword: dietary protein source, exercise recovery, muscle protein synthesis

Outcome measures

Primary outcome

The main study endpoint is the fractional synthetic rate (FSR) of muscle protein synthesis (mixed, myofibrillar, and mitochondrial proteins) from 0-6 hours in the post-prandial period.

Secondary outcome

Secondary endpoints include:

• The fractional synthetic rate (FSR) of muscle protein synthesis (mixed, myofibrillar, and mitochondrial proteins) from 0-2 and 2-6 hours in the post-prandial period.

• Plasma free phenylalanine enrichment (expressed as MPE)

• Plasma free tyrosine enrichment (expressed as MPE)

• Plasma total phenylalanine (expressed as µmol/L)

• Plasma total tyrosine (expressed as µmol/L)

• Total plasma amino acids (AAmax [µmol/L])

• Plasma glucose (glucosemax [mmol/L])

• Plasma insulin (insulinmax [mU/L])

Study description

Background summary

Nutrition plays a key role in facilitating the skeletal muscle adaptive response to exercise training, thereby modulating muscle reconditioning. A

single bout of exercise stimulates both muscle protein synthesis and, to a lesser extent, muscle protein breakdown. However, post-exercise protein balance will remain negative in the absence of food intake. Dietary protein ingestion stimulates skeletal muscle protein synthesis, inhibits protein breakdown and, as such, stimulates muscle protein accretion following both resistance as well as endurance type exercise. This facilitates the skeletal muscle adaptive response to each successive exercise bout, resulting in more effective muscle reconditioning.

Though it has been well established that dietary protein ingestion effectively stimulates muscle protein synthesis rates both at rest and following exercise, there is much less information on the amount and type of dietary protein that should be ingested to maximize post-exercise muscle protein synthesis rates. Moore et al. reported that post-exercise muscle protein synthesis rates increase with the ingestion of greater amounts of protein, reaching maximal stimulation after ingesting 20 g (egg) protein. This has led to the general advice to take at least 20 g of a high quality protein after a workout. Improvements in post-exercise protein balance and/or greater muscle protein synthesis rates have been reported following the ingestion of various types of protein, including whey protein, casein protein, soy protein, casein protein hydrolysate, egg protein, and whole-milk and/or fat-free milk. To date, only a few studies have directly compared the post-exercise muscle protein synthetic responses to the ingestion of different types of protein. For example, Tang and colleagues compared the muscle protein synthetic response between whey, casein, and soy protein after resistance exercise in young men and demonstrated that whey stimulated greater muscle protein synthesis rates than both casein and soy. Soy was intermediate in that it was less effective than whey protein but more effective than casein at stimulating muscle protein synthesis after resistance exercise. Previous work from our own lab corroborated the findings of Tang and colleagues by demonstrating that whey protein stimulated greater muscle protein synthesis rates in older men than did casein or casein hydrolysate. However, bovine milk protein (which is 80% casein and 20% whey protein) seems to offer an anabolic advantage over soy protein after resistance exercise. To date, no studies have directly compared milk protein to its isolated constituent proteins whey and casein. The differences in the muscle protein synthetic response to the ingestion of various protein sources can be attributed to differences in protein digestion and absorption kinetics as well as amino acid composition. Both protein digestion and absorption kinetics as well as leucine content seem to be instrumental to stimulate muscle protein synthesis, but are certainly not the only factors responsible for maximizing post-exercise muscle protein synthesis rates. Whereas casein and whey protein have been applied in research to investigate the impact of slowly versus more rapidly digestible proteins, this work is certainly not representative of the digestion and absorption kinetics of milk protein. Furthermore, recent work in our group has extended on previous work by showing that the anabolic response to casein ingestion can be increased by co-ingesting free leucine. We hypothesize that milk protein is as effective as whey protein at promoting muscle protein synthesis during recovery from exercise. Furthermore, we

speculate that co-ingestion of free leucine with a typical plant based protein (soy protein) may further augment post-exercise muscle protein synthesis rates making soy protein equally effective as milk protein to stimulate post-exercise muscle protein synthesis.
Besides rehydration, a rapid restoration of depleted muscle glycogen stores is an important target following completion of a single bout of exercise. Therefore, recovery drinks generally contain both carbohydrate and protein. Interestingly, the combined ingestion of protein and carbohydrate have been shown to accelerate muscle glycogen repletion when less than optimum amounts of carbohydrate are ingested during the first few hours of post-exercise recovery. Furthermore, though carbohydrate ingestion does not modulate post-exercise muscle protein synthesis rates they may attenuate the post-exercise rise in muscle protein breakdown rates, thereby further improving protein balance.
It is evident that more applied work is needed to define the post-exercise anabolic properties of various protein sources when ingested in combination with carbohydrate, as the combined ingestion will modulate both digestion and absorption kinetics of the various protein sources and the associated post-prandial hormonal response. This project compares the muscle protein synthetic response to the ingestion of various dairy protein sources (whey, casein, and milk protein) as well as a plant-based protein (soy protein) when ingested with carbohydrate during recovery from an intense bout of resistance type exercise in young males. Furthermore, we will assess the impact of adding free leucine to a soy protein plus carbohydrate beverage as a strategy to further augment the properties of soy protein to increase post-exercise muscle protein synthesis rates. The findings from the proposed work will yield new insights within the field of applied exercise physiology by defining the best protein source to consume with carbohydrate to maximize the skeletal muscle adaptive response to exercise training.

Study objective

Overall Objective: To define the properties of whey, casein, milk protein, as well as soy protein with and without additional leucine to augment post-exercise muscle protein synthesis when co-ingested with a carbohydrate containing recovery drink.

Study arm 1
Objective: To compare the anabolic properties of a carbohydrate placebo vs. whey, casein, and milk protein when co-ingested with a carbohydrate containing beverage on post-exercise muscle protein synthesis rates in young males.

Study arm 2
Objective: To compare the anabolic properties of whey protein, soy protein, and soy protein with additional free leucine to match that found in whey protein when ingested with a carbohydrate containing beverage on post-exercise muscle protein synthesis rates in young males.

Study design

STUDY DESIGN
The present study employs a parallel group design. In total, 96 (6 groups of 12 subjects spread over 2 study arms) healthy young male subjects will be included in the study. Subjects will be randomly assigned (using permuted blocks) to consume either carbohydrate, or carbohydrate with whey, casein, milk protein, soy protein, or soy protein and additional leucine. During the test day, subjects will perform a bout of concurrent training involving resistance exercise (press, extensionl) and endurance exercise (cycle ergometry) and immediately afterwards consume their respective nutritional treatment. Study arm 2 will be performed in parallel with study arm 1 to allow the whey protein group in Study arm 1 to act as a positive control group in Study arm 2.

Screening & pre-testing
Subjects will participate in one screening session in which leg volume, blood pressure, body weight and composition (DEXA) will be assessed. Subjects will be asked to fill in a medical questionnaire inquiring about their general health, medical history, use of medication and sports activities. Additionally, all subjects will participate in an orientation session for familiarization with the exercise equipment. Following the orientation subjects will undergo strength testing to determine their single repetition maximum on each exercise followed by a test of maximum wattage on a cycle ergometer.
Body weight and height will be assessed, as well as body fat composition (percentage) via a Dual Energy X-ray Absorptiometry (DEXA) scan. In the event of an unexpected medical finding during the screening, subjects will always be notified. If a subject does not want to receive this notification he cannot participate in the study. Following the DEXA, subjects will undergo a blood pressure reading which involves placing an inflatable cuff around the upper the. Blood pressure will be taken consecutaively 3 times and the average will be recorded. During the familiarization session with the exercise equipment, proper lifting technique will be demonstrated for knee extension exercise. Guided-motion exercise machines will be used to promote proper form and for the subject*s personal safety. Prior to the determination of the subjects* one repetition maximum (1RM), they will perform 2 sets of each respective exercise for 10 repetitions on the exercise machine at a light load. Thereafter, the load will be increased after each successful lift until failure. A 2 minute rest period will be allowed between attempts. A repetition will be considered valid if the subject uses proper form and is able to complete the entire lift in a controlled manner without assistance. Maximum wattage will determined during an incremental test to volitional fatigue. Subjects will commence cycling at a workload equivalent to 2 W/kg for 150 sec. Thereafter, the workload will be increased by 25 W every 150 sec until volitional fatigue, defined as the inability to maintain a cadence > 70 revolutions/min.

Diet and activity prior to testing

All subjects will consume a standardized dinner the evening before the test day. This standardized dinner is an *Aviko maaltijdpannetje* and will be purchased at a regular supermarket in Maastricht. The expiration date from the manufacturer will be checked. Meanwhile, the meals will be stored in an appropriate freezer of the *dietary-kitchen* at the department of Human Biology. The subjects will receive the meal after the screening test in a thermal bag. The precise composition and preparation methods are described on the label of the product (Appendix D4.1). The subjects will be instructed to store the meal in a freezer until preparation and to prepare the meal themselves according to the instructions on the label. All subjects will be instructed to refrain from any sort of heavy physical exercise and to keep their diet as constant as possible 3 d before the test day. In addition, subjects will be asked to record their food intake for 48 h before the start of the test day in a food diary that will be provided during the screening.

Experimental test day
Each subject will participate in 1 experimental test day lasting ~8.5 h. During the test day, subjects will perform a single bout of concurrent exercise (both endurance and resistance) and will ingest their randomly assigned nutrient treatment beverage. The use of [13C6] phenylalanine and (3,5-D2)-tyrosine will allow us to assess mixed, myofibrillar, and mitochondrial protein synthesis rates (FSR) during the post-exercise recovery period in an in vivo human setting.
Protocol
An outline of the study protocol is shown in Figure 1. At 8.00 am, following an overnight fast, subjects will arrive at the laboratory by car or public transportation. Subjects will rest in a supine position and a Teflon catheter will be inserted into an antecubital vein for intravenous stable isotope infusion. A second Teflon catheter will be inserted in a heated dorsal hand vein of the contralateral arm and placed in a hot-box (60°C) for arterialized blood sampling. Following basal blood collection (8 mL; t=-150 min), the plasma phenylalanine and tyrosine pool will be primed with a single intravenous dose of tracer and a continuous tracer infusion will commence. Arterialized blood samples (8 mL) will then be drawn at t= -90, -30, and 0. Starting at -45, subjects will perform a bout of resistance exercise consisting of two different leg exercises (seated knee extension, supine leg press) on a guided-motion exercise machine for 4 sets at a load they can lift for 10 - 12 repetitions. Subjects will be allowed to rest 2 minutes in between each exercise set and the load will be adjusted to maintain the desired 10-12 repetitions. Immediately following the resistance exercise, subjects will perform a bout of endurance-type exercise on a cycle ergometer at ~60% of maximum wattage for 30 minutes. Immediately after the exercise bout subjects will return to the resting supine position and arterialized blood sample will be drawn. Afterwards, a muscle biopsy will be collected (t=0 min). Subjects will then receive their respective nutritional treatment (t= 0). Arterialized blood samples (8 ml) will be collected at t= 15, 30, 60, 90 and 120 min during the postprandial (fed) period. At 120 min in the postprandial period, the second

muscle biopsy will be taken from the same leg as the last biopsy but from a different incision. Subsequently, arterialized blood samples (8 ml) will be collected at t=150, 180, 240, 300, and 360 min. Finally, at 360 min a third muscle biopsy will be taken. In total, three muscle biopsies will be taken through three separate incisions during the trial. The muscle biopsies (immediately, 2 h, and 6 h after exercise) will allow us to measure the temporal response of muscle protein synthesis between the different consumed protein sources after exercise. It is generally assumed that *peak* stimulation of muscle protein synthesis rates is more meaningful in predicting phenotypic outcomes (muscle hypertrophy). However, peak muscle protein synthesis rates may appear at different time points depending on the protein source consumed. Obtaining a muscle biopsy at 2 h post-exercise will allow us to determine peak muscle protein synthesis rates between the different consumed protein sources. However, resistance exercise-induced muscle protein synthesis rates can extend beyond this 2 h time point and thus obtaining an third muscle biopsy at 6 h will also allow us to obtain physiological relevant information with regards to whether there are differences between protein sources in the duration of the anabolic response to combined resistance and endurance exercise.

Intervention

Randomized, double-blind, parallel group design in which subjects are randomly assigned to one of the 6 different nutrition treatment groups: carbohydrate (placebo), whey protein, casein protein, milk protein, soy protein, soy protein + additional free leucine.

Study burden and risks

DUAL-ENERGY X-RAY ABSORPTIOMETRY (DXA) SCAN
A dual-energy x-ray absorptiometry (DXA) scan will be used to asses the amount of muscle in your whole body. This is a painless non-invasive procedure that uses a small amount of radiation to assess how much fat, bone, and lean tissue you have in your entire body. This procedure will take place at the McMaster University Medical Centre and you will be scanned once before starting the study.

Potential Risks. The DXA scan involves exposure to radiation. The radiation dose is ~0.001 microSieverts, which is about the amount of radiation an average person receives every 24 hours from natural radiation in our environment (i.e., from the sun, television and computer screens etc).

MUSCLE BIOPSY SAMPLING
This procedure involves the removal of a small piece of muscle tissue using a sterile hollow needle. A trained investigator will clean an area over your quadriceps muscle (vastus lateralis) with antiseptic solution and then inject a small amount of local anaesthetic ("freezing") into and under your skin. The

investigator will then make a small incision (~4-5 mm) in your skin in order to create an opening through which to put the biopsy needle into your thigh muscle. There will be a small amount of bleeding from the incision, but this will be minimal. The investigator will then quickly cut off a very small piece of muscle (~150 mg; about the size of half of a pea or an eraser on the end of a pencil) and remove the needle from your leg. During the time that the sample is being taken (~5 sec), you may feel the sensation of deep pressure in your thigh and on some occasions this is moderately painful. However, the discomfort very quickly passes and you are quite capable of performing exercise and daily activities. In some cases, you may experience dizziness, cold sweat, increased heart rate or difficulty in breathing as a reaction to the needle prick when anaesthetizing your thigh or the insertion of the biopsy needle, similar to the reactions that you might have when giving blood. However, these sensations will subside fairly quickly with rest and elevation of your feet. If you do feel these sensations or in any way faint at all then please let the investigators know as soon as possible.

Following the biopsies, the incisions will be closed with a steri-strip, and wrapped with a tensor bandage. You should refrain from excessive muscle use for the remainder of the day. Once the anaesthetic wears off, your leg may feel tight and often there is the sensation of a deep bruise or "Charlie Horse". Analgesics (pain killers) such as Tylenol or Ibuprofen (Motrin) are acceptable if you experience significant pain associated with the biopsy. It is also beneficial to periodically apply an ice pack to the biopsy site during 24-48 hours after the biopsy, as this will help to reduce any swelling and any residual soreness. The following day your leg may feel uncomfortable when going down stairs. The tightness in the muscle usually disappears within 2 days and subjects routinely begin exercising at normal capacity within a day. In order to allow the incisions to heal properly and minimize any risk of infection, you should avoid prolonged submersion in water for 2-3 days. Daily showers are acceptable so long as you pat the area dry following the shower, but baths, swimming, saunas, or any water immersion should be avoided for at least 5 days following the biopsy procedure.
Potential Risks. The biopsy technique is routinely used in physiological research, and complications are rare provided that proper precautions are taken. However, there is a risk of internal bleeding at the site of the biopsy, which can result in bruising and temporary discoloration of the skin. On occasion a small lump may form under the site of the incision, but this normally disappears within 2-3 weeks. As with any incision there is also a risk of infection, however this risk is virtually eliminated through proper cleansing of the area and daily changing of wound coverings. If the incision does not heal within a few days or you are in any way concerned about inflammation or infection (usually this means that the incision site is red, hot, swollen and/or itchy), please remove the stitch, clean the cut and contact us immediately. In very rare occasions, there can be damage to a superficial sensory nerve, which will result in temporary numbness in the area. There is also an extremely remote chance that you will be allergic to the local

anaesthetic (lidocaine); the real incidence of lidocaine allergy is unknown.

In past experience with healthy young subjects, approximately 1 in 2,200 have experienced a local skin infection; 1 in 500 have experienced a small lump at the site of the biopsy (in all cases this disappeared within ~2-3 weeks using local massage); 1 in 1,500 have experienced a temporary loss of sensation in the skin at the site of incision (an area of numbness about the size of a quarter which lasted up to 3-4 months), and 1 in 30 have experienced bruising around the site of incision which lasted for ~4-5 days. To the best of our knowledge, no adverse reactions have been reported by older subjects who have undergone the muscle biopsy procedure. While there is also a theoretical risk of damage to a small motor nerve branch (that is used to allow your muscle to move) of the medial vastus lateralis, this has never been seen in over 9,500 biopsies performed by the investigators at McMaster University in Canada (Suction-modified Bergström muscle biopsy technique: experience with 13,500 procedures. Tarnopolsky MA, Pearce E, Smith K, Lach B. Muscle Nerve. 2011 May;43(5):717-25). Hence, the risk of damaging a small motor nerve branch is remote.

FOREARM VENOUS CATHETERIZATION & STABLE ISOTOPE-LABELLED AMINO ACID INFUSION
A study investigator familiar with this procedure will first place a needle into veins in both of your arms (blood vessels that takes blood from your arms to your heart) to take a sample of your blood. The needles will be placed in what are called your antecubital veins. In this experiment, the total amount of blood taken from the antecubital veins will be 112 ml, which is ~1/5 of a cup of blood. You will then receive, through a small catheter placed in your arm, an infusion (slow measured amount) of an amino acid (a small component of protein) solution. The amino acid will be dissolved in saline (a salt solution similar to your blood). The amino acid will be labelled with a stable isotope of carbon, hydrogen, or nitrogen. An isotope is slightly heavier form of these elements. Since the isotope is stable (i.e., non-radioactive) it poses no health risk to you due to radioactive exposure. In addition, a certain fraction of all of the carbon, hydrogen, and nitrogen within your body is already in the same form as that of the stable isotope. Hence, the infusion of the stable isotope-labelled amino acid will simply result in a slight increase in the amount of stable isotope within your body; we refer to this as "enriching" the amount of stable isotope within your body. This enrichment will not remain high, however, and will be back to pre-infusion levels within a few days. All of the infused solutions are prepared under sterile conditions and are filtered through a very selective filter prior to entering your body. All solutions that enter your body do not contain, except for the amino acid, anything that will affect your health.

Potential Risks. The insertion of catheters for blood sampling is a common medical practice and involves few risks if proper precautions are taken. The catheters are inserted under completely sterile conditions; however, there is a theoretical risk of infection. There is also a chance of internal bleeding if

adequate pressure is not maintained upon removal of the catheter. This may cause some minor discomfort and could result in bruising/skin discoloration, which could last for 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 catheter placements. Despite all precautions, there is a theoretical risk (less than 1 in 1,000,000) that you could have a rapid drop in blood pressure due to some small bacterial contamination of the infusion solution (infusate). This has never occurred in our experience.

BENEFITS:
There are no direct benefits to the subjects.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

Male
• Healthy and physically active (as determined by medical and activity questionnaire)
• 20-30 years of age
• Having given informed consent

Exclusion criteria

• Having any identified metabolic or intestinal disorders
• Tobacco use
• Aspirin use in the 4 days prior to the experimental trial
• Consumption of prescription medications or any performance enhancing agent
• Inability to endure the strenuous exercise bouts e.g. injuries
• Alcohol intake during the 48 hours prior to each of the testing days
• Currently participating or having participated in another clinical trial during the last 4 weeks prior to the beginning of this study

Study design

Design

Study type:	Interventional
Intervention model:	Parallel
Allocation:	Randomized controlled trial
Masking:	Double blinded (masking used)
Control:	Placebo
Primary purpose:	Treatment

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	19-03-2015
Enrollment:	96
Type:	Actual

Ethics review

Approved WMO Date:	08-01-2015
Application type:	First submission
Review commission:	METC academisch ziekenhuis Maastricht/Universiteit Maastricht, METC azM/UM (Maastricht)
Approved WMO Date:	08-06-2015
Application type:	Amendment
Review commission:	METC academisch ziekenhuis Maastricht/Universiteit Maastricht, METC azM/UM (Maastricht)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

ID: 23128
Source: Nationaal Trial Register
Title:

In other registers

Register	ID
CCMO	NL49732.068.14
OMON	NL-OMON23128