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Protein intake, endurance exercise, and muscle protein synthesis

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Registration date 17/01/2025	**Overall study status** Ongoing	[ ] Statistical analysis plan [ ] Results
Last Edited 20/12/2024	**Condition category** Other	[ ] Individual participant data [X] Record updated in last year

Plain English summary of protocol

Background and study aims
Proteins play important structural and regulatory roles within the body. Active individuals have greater dietary protein requirements than sedentary individuals. Sport nutrition research has traditionally focused on the protein needs of strength-training athletes. Far less is known about the protein needs of endurance athletes. Endurance training likely increases protein requirements due to the need to replace amino acids lost to oxidation and other metabolic processes, and to support increased protein turnover and remodelling of skeletal muscle towards a more aerobic phenotype (i.e., training adaptation). Another factor affecting skeletal muscle protein metabolism is the timing of protein intake. Eating a protein-rich meal or snack every 3-4 hours supports the highest rates of muscle protein synthesis throughout the day. However, endurance athletes often go many hours without consuming protein due to the length of time spent training and not eating, which could have implications for muscle protein metabolism and the effectiveness of training. The present study will determine the effect of increased protein intake (1.7 g vs 1.0 g/kg/day) on muscle protein synthesis rates during a 24-hour period in which prolonged (4 hours) cycling exercise is performed. It will also be determined whether muscle protein synthesis rates are influenced by the timing of extra protein intake (during vs after exercise). This research will expand our understanding of protein and amino acid metabolism during endurance exercise and recovery, with the potential to impact nutritional strategies and dietary recommendations for those involved in recreational endurance sports.

Who can participate?
Healthy non-smoking males aged 30-59 years who are experienced cyclists or triathletes with predicted or actual 100 km times <5 hours and with no history of cardio-metabolic disease.

What does the study involve?
The study will consist of four laboratory visits: a preliminary visit 2-3 weeks before the main experiment, then three further visits on consecutive days.

Visit 1 – Consenting, screening and baseline testing
Upon arrival, the study will be fully explained to the participant. They will then be asked to sign an Informed Consent Form if they have decided to take part. A general health questionnaire will

be completed, and body height and body mass will be measured and recorded. If they complete the questionnaire and meet the eligibility requirements, participants will then have their aerobic fitness determined using a test known as a VO2max test, which will take place on a stationary bicycle. The test starts at a very easy intensity and gets harder every 3 minutes, and participants are asked to keep cycling for as long as they can (typically this takes between 20 and 30 minutes). During the test, participants will be wearing a facemask connected to a machine for breath-by-breath gas analysis (oxygen and carbon dioxide) that will measure how much oxygen they are using to sustain the exercise. If their test results meet the set criterion, they will be invited for subsequent visits. If they are eligible to take part, after a brief rest, they will cycle for a further 20 minutes at a workload of 50% of their individually assessed maximum aerobic power output to familiarise with the intensity of exercise for the main experiment.

Pre-trial standardisation
Diet and activity will be standardised for 5 days before the experiment.
Diet - Participants will be provided with food packages to consume each day. They will be asked to only consume the food and drinks provided, but they can drink as much plain water as they like. They are not permitted to consume any diet/sport nutrition supplements that have not been approved by an investigator. All food will be prepared by the research team at the study site kitchen facility. All food will be pre-weighed, pre-packed, and labelled with clear instructions for storage, cooking, and consumption, including when they should consume each item.

Activity - Three to five days out from the experiment (days -5, -4, -3), participants can exercise normally, but daily training should not exceed 2 hours in total, and the training sessions should not include high-intensity efforts. For the next 2 days (days -2, -1), participants will be asked to refrain from all exercise training and any demanding physical activity.

Visit 2 - Heavy water (D2O) ingestion
Participants will arrive at the laboratory in the afternoon before the main experiment. A saliva sample will be collected by having the participant spit into a small test tube. Next, they will be provided with the heavy water to consume. The total tracer dose will be split into four smaller doses of equal quantities to be consumed at 1-hour intervals. The first two doses will be consumed in the laboratory, in the presence of an investigator. The final two doses will be consumed at their home. They will be provided with instructions for the timing of ingestion and a sheet to record compliance.

Visit 3: Experimental Trial - Day 1
Participants will arrive at the laboratory in the morning (7.00-9.00 am) after a 12-hour overnight fast. When they arrive, they will be asked to go to the toilet. Next, their body mass will be measured and recorded. Thereafter, they will be asked to provide a saliva sample by spitting into a tube. Then, their leg soreness will be measured using a visual analogue scale. Next, an indwelling intravenous cannula will be fitted into a vein of one arm. A muscle biopsy sample will then be collected from their thigh under local anaesthetic using a 5mm diameter biopsy needle. Once the muscle biopsy has been obtained, they will be provided with a standardised breakfast to consume. One hour after finishing breakfast, they will begin cycling at 50% maximal aerobic power output and cycle for 4 hours, with brief, 5-minute breaks after the first and second 1.5 hours of cycling. During these breaks they will have the opportunity to use the toilet and stretch. Throughout exercise, their heart rate will be monitored continuously, and they will be asked to rate their perceived exertion (every 30 min) and gut comfort (every 60 min). Oxygen consumption and carbon dioxide production will be determined every 30 minutes by having them breathe through a facemask connected to a machine for breath-by-breath gas analysis. Blood samples will be collected via the intravenous cannula at 30-minute intervals. Fluid in the form of a sport drink will be ingested at a rate of 10 ml/kg/hour. After completing the 4-hour

cycle, participants will rate their leg muscle soreness, and undergo another muscle biopsy. Thereafter, the cannula will be removed. A saliva sample will be provided before they consume lunch and a recovery drink. Finishing lunch marks the end of the laboratory visit. Before leaving, they will be provided with test tubes for home saliva sampling, a post-trial instruction sheet related to biopsy aftercare, and a standardised evening meal and recovery drink to consume at a fixed time of the day.
The sports drinks provided during exercise and recovery will contain either carbohydrate only or carbohydrate plus protein, depending on the trial condition. Participants will be randomly assigned to one of 3 trial conditions:
1. Control
2. Extra protein during exercise
3. Extra protein after exercise

Visit 4: Experimental Trial - Day 2
Participants will arrive at the laboratory in the morning (same time as the previous day) after an overnight fast. After voiding and being weighed, they will provide a saliva sample. They will then rate their leg muscle soreness. Next, a blood and muscle sample will be obtained. Lastly, they will be asked whether they suspected which nutritional treatment they were assigned to. After this, they will be provided with a breakfast to consume before they are free to leave the laboratory.

What are the possible benefits and risks of participating?
Throughout the study participants will undertake a range of tests that will generate information that they might find interesting for their own knowledge and application to training. The most obvious risks will involve the muscle biopsy (tissue sample), lidocaine administration, exercise testing, blood sampling and stable isotope ingestion.

Where is the study run from?
University of Birmingham (UK)

When is the study starting and how long is it expected to run for?
January 2025 to August 2026

Who is funding the study?
Volac International (UK)

Who is the main contact?
Jil Weber, jxw1657@student.bham.uk

Contact information

Type(s)
Public

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Type(s)
Scientific, Principal Investigator

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Additional identifiers

EudraCT/CTIS number
Nil known

IRAS number
345203

ClinicalTrials.gov number
Nil known

Secondary identifying numbers
RG_24-050

Study information

Scientific Title
The impact of protein quantity and timing on muscle protein synthesis during endurance exercise and recovery

Study objectives

Increased protein intake (1.7 vs 1.0 grams/kg body mass/day) will result in higher rates of muscle protein synthesis during a 24 hour period in which prolonged cycling exercise is performed.

The timing of additional protein intake (during vs after exercise) will not affect this response.

Ethics approval required
Ethics approval required

Ethics approval(s)
Approved 07/11/2024, East Midlands - Nottingham 2 Research Ethics Committee (Redman Place, Stratford, E20 1JQ, United Kingdom; +44 (0)207 104 8009; nottingham2.rec@hra.nhs.uk), ref: 24/EM/0210

Study design
Single-centre single-blinded randomized controlled trial

Primary study design
Interventional

Secondary study design
Randomised parallel trial

Study setting(s)
Laboratory

Study type(s)
Other

Participant information sheet
Not available in web format, please use contact details to request a participant information sheet.

Health condition(s) or problem(s) studied
Endurance cycling exercise and recovery

Interventions
The study will consist of four laboratory visits: a preliminary visit 2-3 weeks before the main experiment, then three further visits on consecutive days.

Visit 1 – Consenting, screening and baseline testing
Upon arrival, the study will be fully explained to the participant. They will then be asked to sign an Informed Consent Form if they have decided to take part. A general health questionnaire will be completed, and body height and body mass will be measured and recorded. If they complete the questionnaire and meet the eligibility requirements, participants will then have their aerobic fitness determined using a VO2max test, which will take place on a stationary bicycle. The test starts at a very easy intensity and gets harder every 3 minutes, and participants are asked to keep cycling for as long as they can (typically this takes between 20 and 30 minutes). During the test, participants will be wearing a facemask connected to a machine for breath-by-breath gas analysis (oxygen and carbon dioxide) that will measure how much oxygen they are using to sustain the exercise. If their test results meet the set criterion, they will be invited for subsequent visits. If they are eligible to take part, after a brief rest, they will cycle for a further

20 minutes at a workload of 50% of their individually assessed maximum aerobic power output to familiarise themselves with the intensity of exercise for the main experiment.

Pre-trial standardisation
Diet and activity will be standardised for 5 days before the experiment.
Diet - Participants will be provided with food packages to consume each day. They will be asked to only consume the food and drinks provided, but they can drink as much plain water as they like. They are not permitted to consume any diet/sport nutrition supplements that have not been approved by an investigator. All food will be prepared by the research team at the study site kitchen facility. All food will be pre-weighed, pre-packed, and labelled with clear instructions for storage, cooking, and consumption, including when they should consume each item.

Activity - Three to five days out from the experiment (days -5, -4, -3), participants can exercise normally, but daily training should not exceed 2 hours in total, and the training sessions should not include high-intensity efforts. For the next 2 days (days -2, -1), participants will be asked to refrain from all exercise training and any demanding physical activity.

Visit 2 - Heavy water (D_2O) ingestion
Participants will arrive at the laboratory in the afternoon before the main experiment. A saliva sample will be collected by having the participant spit into a small test tube. Next, they will be provided with the heavy water to consume. The total tracer dose will be split into four smaller doses of equal quantities to be consumed at 1-hour intervals. The first two doses will be consumed in the laboratory, in the presence of an investigator. The final two doses will be consumed at their home. They will be provided with instructions for the timing of ingestion and a sheet to record compliance.

Visit 3: Experimental Trial - Day 1
Participants will arrive at the laboratory in the morning (7.00-9.00 am) after a 12-hour overnight fast. When they arrive, they will be asked to go to the toilet. Next, their body mass will be measured and recorded. Thereafter, they will be asked to provide a saliva sample by spitting into a tube. Then, their leg soreness will be measured using a visual analogue scale. Next, an indwelling intravenous cannula will be fitted into a vein of one arm. A muscle biopsy sample will then be collected from their thigh under local anaesthetic using a 5 mm diameter biopsy needle. Once the muscle biopsy has been obtained, they will be provided with a standardised breakfast to consume. One hour after finishing breakfast, they will begin cycling at 50% maximal aerobic power output and cycle for 4 hours, with brief, 5-minute breaks after the first and second 1.5 hours of cycling. During these breaks they will have the opportunity to use the toilet and stretch. Throughout exercise, their heart rate will be monitored continuously, and they will be asked to rate their perceived exertion (every 30 min) and gut comfort (every 60 min). Oxygen consumption and carbon dioxide production will be determined every 30 minutes by having them breathe through a facemask connected to a machine for breath-by-breath gas analysis. Blood samples will be collected via the intravenous cannula at 30-minute intervals. Fluid in the form of a sports drink will be ingested at a rate of 10 mL/kg/hour. After completing the 4-hour cycle, participants will rate their leg muscle soreness, and undergo another muscle biopsy. Thereafter, the cannula will be removed. A saliva sample will be provided before they consume lunch and a recovery drink. Finishing lunch marks the end of the laboratory visit. Before leaving, they will be provided with test tubes for home saliva sampling, a post-trial instruction sheet related to biopsy aftercare, and a standardised evening meal and recovery drink to consume at a fixed time of the day.

Group randomisation will be performed using a computer-generated minimisation programme (Bland & Altman, 2005), with participants stratified by age and fitness level (as measured by VO2 max). This approach will help minimise potential imbalances across the three study arms.

The sports drinks provided during exercise and recovery will contain either carbohydrate only or carbohydrate plus protein, depending on the trial condition. Participants will be randomly assigned to one of three trial conditions:
1. Control: The diet will contain 1 g per kg body mass per day which will be provided in meals served as breakfast, lunch, and dinner
2. Extra protein during exercise: The control diet will be supplemented with an extra 0.7 g per kg body mass provided as whey protein consumed during exercise
3. Extra protein after exercise: The control diet will be supplemented with an extra 0.7 g per kg body mass provided as whey protein consumed during the post-exercise recovery period

Visit 4: Experimental Trial - Day 2
Participants will arrive at the laboratory in the morning (same time as the previous day) after an overnight fast. After voiding and being weighed, they will provide a saliva sample. They will then rate their leg muscle soreness. Next, a blood and muscle sample will be obtained. Lastly, they will be asked whether they suspected which nutritional treatment they were assigned to. After this, they will be provided with a breakfast to consume before they are free to leave the laboratory.

Intervention Type
Other

Primary outcome measure
Mixed muscle protein synthesis is determined by measuring the tracer enrichment of biopsy samples taken at baseline, post exercise and 24 h.

Secondary outcome measures
1. Blood and muscle intracellular amino acid concentrations measured using LC-MS/MS. Blood samples analysed at baseline, every 30 min throughout exercise, and at 24 h. Intracellular samples analysed at baseline, post exercise, and at 24 h.
2. Blood substrates and hormones measured using colorimetric assays and ELISA. Samples analysed at baseline, every 30-60 min throughout exercise, and at 24 h.
3. Muscle glycogen content measured as free glucose after acid hydrolysis. Samples analysed at baseline, post exercise, and at 24 h.
4. Perceived muscle soreness measured using visual analogue scales at baseline, post exercise, and at 24 h.
5. Blood markers of muscle damage measured using colorimetric assays. Samples analysed at baseline, post exercise, and at 24 h.
6. Self-rated gut comfort measured using visual analogue scales every 60 min during exercise
7. Rating of perceived exertion (RPE) measured using standard 6-20 scale. Recorded every 30 min throughout exercise.
8. Substrate oxidation measured using indirect calorimetry every 30 min during exercise

Overall study start date
01/10/2023

Completion date
28/08/2026

Eligibility

Key inclusion criteria
1. Men
2. Aged 30-59 years
3. Experienced cyclists or triathletes with predicted or actual 100km time <5 hours
4. No known history of cardiac or other underlying conditions that may affect participation in the study
5. Compliance: understands and is willing, able and likely to comply with all study procedures and restrictions
6. Consent: demonstrates understanding of the study and willingness to participate as evidenced by voluntary written informed consent
7. In good general health with no previous history of cardio-metabolic disease
8. Body mass index between 18.5 and 29.9 kg/m2
9. Weight stable (no more/less than 5% change in body mass in past 3 months)
10. Aerobic fitness (VO2max): >44 ml/kg/min 30-39 years, >42 ml/kg/min 40-49 years, >39 mL/kg /min 50-59 years (considered 'good' scores as per ACSM fitness categories for maximal aerobic capacity)

Participant type(s)
Healthy volunteer

Age group
Adult

Lower age limit
30 Years

Upper age limit
59 Years

Sex
Male

Target number of participants
45 (15 per group)

Key exclusion criteria
1. Lactose intolerance
2. Lidocaine allergy
3. Current participation in another scientific/ clinical study
4. Ingestion of deuterium oxide (D2O; 'heavy water') in previous 12 months
5. Bleeding disorder/s
6. Current or previous smoker (to include vaping) – within last 5 years
7. Poor recent health as indicated in the general health questionnaire (including musculoskeletal injury)
8. Existence of food allergies and/or intolerances that may affect participation in the study
9. Following a vegetarian or vegan diet
10. Engaging in uncommon eating practices (e.g. sustained periods of fasting)
11. Following a low dietary carbohydrate (high fat and/or high protein) lifestyle

12. Habitual protein intake <0.8 g/kg body mass/day or >2.4 g/kg body mass/day
13. Use of any medication or supplement known to affect muscle protein metabolisms (e.g., beta-blockers, corticosteroids, analgesics, or non-steroidal anti-inflammatories)

Date of first enrolment
06/01/2025

Date of final enrolment
30/06/2026

Locations

Countries of recruitment
England

United Kingdom

Study participating centre
University of Birmingham
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Sponsor information

Organisation
University of Birmingham

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Sponsor type
University/education

Website
http://www.birmingham.ac.uk/index.aspx

ROR
https://ror.org/03angcq70

Funder(s)

Funder type
Industry

Funder Name
Volac International

Results and Publications

Publication and dissemination plan
Planned publication in a peer-reviewed journal in 2027. There are no plans to publish additional files.

Intention to publish date
28/08/2027

Individual participant data (IPD) sharing plan
The datasets generated during and/or analysed during the current study are/ will be available upon request from Dr Carl Hulston (c.hulston@bham.ac.uk) based on a reasonable request and in line with the ethics approval.

IPD sharing plan summary
Available on request