REDUCING EARLY ATROPHY WITH LEUCINE DURING IMMOBILIZATION OF SKELETAL MUSCLE

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Primary Objectives:1. Determine the impact of short-term (3 days) unilateral lower-limb immobilization on *cumulative* rates of MPS in younger and older adults.2. Determine whether leucine supplementation can prevent declines in cumulative MPS and...

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

Summary

ID

NL-OMON45771

Source
ToetsingOnline

Brief title
REALISM

Condition

- Protein and amino acid metabolism disorders NEC
- Muscle disorders

Synonym
Muscle Protein Turover; Muscle Protein Synthesis and Breakdown

Research involving
Human

Sponsors and support

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

Intervention

Keyword: Immobilization, Leucine, Muscle Protein Breakdown, Muscle Protein Synthesis

Outcome measures

Primary outcome

Main study parameter/endpoint

The main study endpoint is cumulative FSR as a measure of muscle protein synthesis rates (MPS) based on the oral tracer deuterium oxide. In order to determine cumulative FSR, the following parameters will be measured via GC-C-IRMS and GCMS respectively:

* Muscle protein-bound L-[2,3,3,3-2H4]-alanine enrichment (expressed as MPE)

* Plasma free L-[2,3,3,3-2H4]-alanine enrichment (expressed as MPE)

* Saliva 2H2O enrichment (Expressed as APE)

Secondary outcome

Secondary study parameters/endpoints

Secondary endpoints include:

* Quadriceps whole-muscle CSA as assessed via CT scan.

* Plasma, muscle free, and muscle protein-bound L-[ring-13C6]-phenylalanine enrichment.

* Fractional breakdown rates (FBR) of muscle protein based on 3,3-D2 phenylalanine tracer dilution in plasma and muscle.

* Fractional synthesis rates (FSR) of muscle protein based on L-[ring-13C6]-phenylalanine tracer incorporation into bound muscle protein.

* Activation of signaling molecules regulating muscle protein synthesis and breakdown (specified in 8.3.6.5) will be established via Western blots.

Quantitative Real-Time PCR Analysis of MAFbx/Atrogin-1, MuRF1, FoxO and

Ubiquitin Expression will also be performed.

Study description

Background summary

Recovery from injury, illness, and/or disease is associated with periods of skeletal muscle disuse. The inactivity associated with periods of disuse results in a loss of skeletal muscle mass (1), leading to negative health consequences including reductions in strength (1), the onset of insulin resistance (2), a decline in basal metabolic rate (3), and body fat mass accumulation (4). These conditions are associated with pre-mature physical frailty (5), elevated health care costs (5), and an increased risk of mortality (6). Muscle disuse forms a major health concern for the elderly who are already at an increased risk for sarcopenia, or age related losses in skeletal muscle mass and strength (7). Episodic periods of muscle disuse that are common in the elderly are now thought to represent a period of *catabolic crisis* that significantly accelerate the progression of sarcopenia (7). Currently, the elderly (age 60 y or >) make up the fastest growing age group, with projections to reach ~21% of the world*s population by the year 2050 (8). Population studies demonstrate that sarcopenia affects >20% of people age 60-70, and ~50% of people >75 (9). Direct healthcare costs attributable to sarcopenia in the USA were ~$18.5 billion in the year 2000 (8). Clearly, it is critical to identify strategies that can effectively offset the loss of muscle mass and strength that occur in response to disuse in order to prevent an epidemic of frailty among the elderly, a loss of independence, and to reduce the risk of morbidity/mortality.
Disuse studies in humans have generally been conducted over periods exceeding 10 days in young healthy subjects (10). These studies have shown that 10-42 days of disuse leads to a rate of muscle loss of ~0.5-0.6% of total muscle mass per day, with a decline in muscle strength between 0.3-4.2% per day (10). However, the loss of muscle mass resulting from disuse is most rapid during the early stages of inactivity (7), with substantial muscle loss occurring in as little as 5 days of muscle disuse (11). This is a major problem for the elderly since the loss of muscle mass and function resulting from 14 days of immobilization cannot be regained with 4 weeks of aggressive resistance exercise training (12). To put this loss into perspective, we have shown that only 5 days of immobilization in elderly men results in a ~1.5% decline in quadriceps muscle cross-sectional area (CSA) (13). Extrapolating this to a whole body level, 5 days of bed-rest would result in *1 kg of muscle loss. Thus, even with *80% recovery of lost muscle mass following this period of disuse, *400 g of muscle would be lost following only two short periods of

illness or injury per year. This equates to 0.8% muscle loss per year, contributing largely to the estimated 1-2% yearly muscle loss from age 50 onwards (14). Currently, little is known about the physiological mechanisms that induce muscle atrophy in response to short-term disuse. Muscle mass is determined by the balance between rates of muscle protein synthesis (MPS) and muscle protein breakdown (MPB). When MPB exceeds MPS, the result is a negative net protein balance and muscle loss. Anabolic stimulation by nutrients, particularly amino acids, is a powerful modulator of skeletal muscle accretion and is a fundamental process for maintenance of skeletal muscle mass. However, declines in basal and postprandial MPS have been reported after 5-42 days of bed rest and lower limb immobilization (10), however whether changes in MPB also occur is unclear given the lack of in vivo assessments of dynamic MPB during disuse. In the absence of direct measures of MPB rates, studies have looked for evidence of an up-regulation of the ubiquitin-proteasome system following disuse as a proxy of increased MPB (10), as this protein degradation pathway and its component enzymes are activated in a catabolic state. However, inconsistent molecular findings, together with a lack of data on dynamic MPB rates, make it difficult to clearly assess the contribution of MPB to muscle atrophy during disuse. The amino acid leucine is recognized as a unique nutrient regulator of muscle protein metabolism as it serves to stimulate MPS, and inhibit MPB by suppressing proteosomal degradation (15). Leucine appears to inhibit muscle atrophy in animal models, in part, through down-regulation of MPB (15). However there is no information available on the impact of leucine supplementation on dynamic measures of MPS and MPB, markers of the ubiquitin/proteasome pathway, and skeletal muscle mass during muscle disuse in humans.

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Study objective

Primary Objectives:

1. Determine the impact of short-term (3 days) unilateral lower-limb immobilization on *cumulative* rates of MPS in younger and older adults.
2. Determine whether leucine supplementation can prevent declines in cumulative MPS and muscle mass in response to 3 days of immobilization in younger and older adults.
3. Identify whether postabsorptive MPB is elevated and MPS is reduced following 3 days of immobilization-induced disuse in younger adults.
4. Determine whether leucine supplementation can reduce MPB, increase MPS, and attenuate the loss of muscle mass during 3 days of immobilization in younger adults.

Hypothesis:

1. In both younger older adults, 3 days of disuse via unilateral lower-limb immobilization will result in muscle atrophy and coincide with decreased rates of *cumulative* MPS during disuse.
2. In both younger and older adults, leucine supplementation during 3 days of disuse via unilateral lower-limb immobilization will attenuate the decline in cumulative MPS and loss of muscle mass.
3. In younger adults, 3 days of disuse via unilateral lower-limb immobilization will increase postabsorptive MPB rates and decreases postabsorptive MPS rates.
4. In younger adults, leucine supplementation during 3 days of disuse via unilateral lower-limb immobilization will reduce the increase in postabsorptive MPB rates and decline in postabsorptive MPS rates following immobilization.

Study design

Study Design
Twenty-four (12 men and 12 women) younger (18-35 years of age inclusive) and twenty-four (12 men and 12 women) older (60-80 years of age inclusive) subjects will be recruited to undergo 3 days of uni-lateral lower limb immobilization. Subjects will be moderately active, but not currently engaged in a structured exercise program. Exclusion criteria will include: lower limb and/or back injuries, a history of thrombosis/cardiovascular disease, use of anticoagulants, musculoskeletal/orthopedic disorders, structured resistance exercise training, use of corticosteroids, use of protein supplements during the study, presence of diabetes, pregnancy, hormone replacement therapy, third generation oral contraceptives, and use of tobacco products. Inclusion criteria will include: moderately active (see above), male or female age 18-35 or 60-80 years inclusive, and BMI not lower than 18.5 and not higher than 30.0.

Screening
When subjects respond to the advertisement, we will contact them by e-mail/phone and briefly explain the study. We will also provide them with the information brochure and the informed consent form which they can read and discuss with family of friends before deciding whether or not to participate. To assess whether subjects are eligible to participate in this study, we will invite them for a screening visit at the University. Before we start the screening, we will explain the entire experimental trial and answer any potential questions. We will then ask them to read, fill out, and sign the informed consent form. After signing the informed consent form, we will start the screening by going through the medical questionnaire to assess their general health, use of medication, and physical activity. The volunteer will be instructed on how to use crutches and to not bear weight on the casted limb, and to minimize muscle contractions of the upper leg. We will also assess weight, height, and blood pressure. The medical questionnaire will be carefully assessed by the responsible physician before subjects are allowed entry into the study. This visit is expected to last 1.5 hours. After the screening the test days (Test day #1, Test day #2 and Test day #3) will be scheduled if applicable (informed consent signed + suitable based on inclusion/exclusion

criteria). It is aimed to book these Test days as soon as possible, depending on the subjects availability. However scheduling will take into account that subjects need the record their food intake for 3 days and wear the Actical activity monitor for 3 days prior to Test day 1.

Diet and activity prior to testing

All subjects will consume a standardized dinner the evening before the onset of immobilization (Test Day #2). In addition, the younger subjects will consume a standardized dinner the evening before the acute amino acid 13C6 tracer investigation (Test Day #3; Figure 3). This standardized dinner is an *Aviko maaltijdpannetje* (Appendix D4.1) 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 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 also be asked to record their food intake for 3 days immediately before the immobilization period and for 3 days during immobilization period in a food diary that will be provided during the screening (Appendix F2.1). In addition, all subjects will also be asked to fill out an activity log and wear an Actical physical activity monitor (Philips) to record their physical activity patterns for 3 days immediately before the immobilization period and for 3 days during the immobilization period. Finally, all subjects will be instructed to refrain from any sort of heavy physical exercise and to keep their diet as constant as possible for the 3 days immediately before and for 3 days during the immobilization period.

Baseline Testing - Test Day #1

Prior to the onset of the uni-lateral immobilization period, subjects will report to the University for baseline testing. Subjects will arrive at the laboratory at 07.30 am in the fasted state having not consumed anything (except water) since 10.00 pm the previous evening. A baseline blood sample and 3 saliva samples will be obtained to measure background 2H enrichment in plasma and body water. Subjects will then begin the deuterated water (deuterium oxide or 2H2O) loading protocol which requires subjects to ingest 50mL of 70 APE enriched deuterated water 8 times during waking hours, at: 0800, 0930, 1100, 1230, 1400, 1530, 1700, 1830 hr to obtain a plateau enrichment of 2H in body water of ~1-2% (16). The subjects will only be required to remain at the laboratory for the first five doses (at 0800, 0930, 1100, 1230, 1400); the remainder can be ingested at home by the subject (1530, 1700, 1830). Following this loading protocol, subjects will be required to ingest one 50mL dose of 70 APE enriched deuterated water each day upon waking to maintain 1-2 APE enrichment in body water (see section 5.1.2). During this visit, we will also assess whole muscle CSA of both the right and left upper leg via CT scan,

whole-body muscle mass by magnetic resonance imaging (MRI), and body composition by dual-energy X-ray absorptiometry (DEXA). First, a single slice CT scan (IDT 8000; Philips Medical Systems, Best, Netherlands) at 15 cm above the base of the patella will be performed in the Academic Hospital Maastricht (Department of Radiology) to determine cross sectional area (CSA) of the upper leg muscle of both legs. While the subjects are supine with their legs extended and their feet secured, a 3-mm thick axial image will be taken. The scanning characteristics will be as follows: 120kV, 300mA, rotation time of 0.75 sec, and a field of view of 500 mm. The exact scanning position will be measured and marked with a semi-permanent marker for replication. Following the CT scans, each participant will undergo a full body MRI scan with a 3 T MR system (Achieva 3Tx; Philips Healthcare), using a radiofrequency transmit/receive body coil with a receiver bandwidth of 31.25 kHz. Using a turbo spin echo sequence with a proton flip angle of 90 degrees, proton density weighted scans will be acquired. This will create an image characterizing the proton density of each tissue type. The images will be acquired using a matrix size of 320-512 pixels and a 55 cm field of view. Contiguous images of 10 mm thickness with no gap will be taken in four to six sequences of 38-40 images for defined body regions for each participant. Following the whole-body MRI, subjects will undergo a DEXA scan. Subjects will be instructed to lie down on a table and stay motionless for approximately 3 minutes during which the body scan takes place. Performing the above mentioned tests will allow us to characterize the whole muscle CSA of both upper legs and subjects* body composition. In case of an unexpected medical finding, we will inform the subjects and their general practitioner. If a subject does not want to receive this information, he or she cannot participate in this study. This visit is expected to last 6.5 hours.

Onset of Immobilization * Test Day #2

The day after Test Day #1, subjects will arrive at the laboratory at 07.30 am in the fasted state having not consumed anything (except water) since the controlled meal (described above, section 3.2) finished no later than 10.00 pm the previous evening. Subjects will first provide a saliva sample, thereafter a blood sample will be obtained, and subsequently undergo a single biopsy from the vastus lateralis of one leg to determine skeletal muscle protein-bound 2H enrichment immediately prior to the onset of the immobilization period. This biopsy will come from the free leg which will not be immobilized. The muscle biopsy will be obtained from the middle region of the vastus lateralis (15 cm above the patella and approximately 2 cm away from the fascia) by the percutaneous needle biopsy technique (17). Muscle biopsies will be carefully freed from any visible non-muscle tissue and immediately be frozen in liquid nitrogen. Muscle biopsies will be stored at *80 ºC for subsequent analysis. The leg chosen to undergo immobilization will be randomized. Following the biopsy, the opposite leg will be immobilized with a cast from 10 cm above the ankle to half way the upper leg (30 degree flexion of the knee-joint; see Figure 1). The cast will remain on for 3 days. Next, the volunteer will be instructed on how to use crutches and to not bear weight on the casted limb, and to minimize muscle contractions of the upper leg. This protocol has been already

successfully used for previous immobilization studies (METC 09-3-011, METC 11-3-073, METC 12-3-012, METC 12-3-063, METC 13-3-023). A model of uni-lateral lower limb immobilization will allow for comparison of an immobilized and non-immobilized lower limb within the same research subject, allowing each subject to serve as their own internal control (11, 13). Following the fitting of the cast, transport home from the university will be arranged either by car or taxi. This visit is expected to last 2 hours.

During Immobilization

During the 3 days of immobilization, all subjects will be checked upon regularly by a member of the research team. These check-ups will serve to answer any questions/concerns from the participant and to check compliance with the protocol. All subjects are at liberty to seek contact with the researcher and/or physician to discuss any possible problems related to the immobilization or participation in the study.

During immobilization, subjects will continue to ingest 2H2O (50 ml serving 1 x day in the morning) to *maintain* the steady-state enrichment in body water and provide a saliva sample for the determination of 2H enrichment in body water (in the evening). The subjects will provided with plastic salivette vials that contain a cotton swab used to collect the saliva sample. The subject will chew and suck on the cotton swab for 30-60 seconds, until the swab is saturated with saliva. Subsequently, they will place the wet swab back inside the salivette vial and store it in their freezer until the next visit to the University.

During immobilization, subjects will receive either a leucine or carbohydrate (maltodextrine) supplement in a double-blinded manner to ingest with each main meal (breakfast, lunch, dinner). Each supplement serving will amount to 5.0 g leucine or carbohydrate for a total of 15 g per day.

End of Immobilization * Test Day #3

Older Subjects

In the morning, after 3 days (or 72 hours) of immobilization, subjects will come to the university for the post immobilization visit. First, the cast will be removed, however the volunteer will not be allowed to place any weight on the leg. Instead, the subject will be transported in a wheelchair to the CT scanner where a single slice CT scan will be performed as described above. Thereafter, subjects will be transported by wheelchair to the laboratory where they will immediately undergo 2 skeletal muscle biopsies; one from the immobilized and non-immobilized leg respectively. Thus, the biopsy from the leg exposed to immobilization will be collected while the subject is still in the immobilized state (i.e. before they have performed any weight bearing muscle contractions). The two biopsies will allow for the determination of cumulative (over 3 days) measures of muscle protein synthesis rates in both the immobilized and non-immobilized leg based on the change in muscle protein-bound 2H enrichment from the first biopsy taken before the onset of immobilization.

We will also take a blood and saliva sample to measure 2H enrichment in plasma and body water as described above. In total the elderly subjects will have 3 muscle biopsies and 3 blood samples (3 x 8 mL = 24 mL total). This visit is expected to last 2 hours.

Younger Subjects
In the morning, after 3 days (or 72 hours) of immobilization, subjects will come to the university for the post immobilization visit. First, the cast will be removed, however the volunteer will not be allowed to place any weight on the leg. Instead, the subject will be transported in a wheelchair to the CT scanner where a single slice CT scan will be performed as described above. Thereafter, subjects will be transported by wheelchair to the laboratory where they will undergo a primed-constant infusion of L-[ring-13C6] phenylalanine for 5-hours. 2 h after the start of the infusion subject will undergo 2 skeletal muscle biopsies; one from the immobilized and non-immobilized leg respectively. Thus, the biopsy from the leg exposed to immobilization will be collected while the subject is still in the immobilized state (i.e. before they have performed any weight bearing muscle contractions). The two biopsies will allow for the determination of cumulative (over 3 days) measures of muscle protein synthesis rates in both the immobilized and non-immobilized leg based on the change in muscle protein-bound 2H enrichment from the first biopsy taken before the onset of immobilization. We will also take a blood and saliva sample to measure 2H enrichment in plasma and body water as described above. In young subjects, these two biopsies will also serve for the assessment of intracellular free and protein-bound L-[ring-13C6] phenylalanine and baseline 3,3-D2 phenylalanine enrichment in muscle to measure MPB using the tracee-release method (18) (See Figure 3). Immediately after these first two biopsies subjects will undergo a primed-constant infusion of 3,3-D2 phenylalanine for 2-hours, after which this infusion will be stopped to assess the decay in 3,3-D2 phenylalanine in blood and muscle (intracellular free amino acid pool). For this purpose, 40 min after the 3,3-D2 phenylalanine infusion is stopped, a second set of biopsies will be collected from each leg (figure 3) to assess intracellular free and protein-bound L-[ring-13C6] phenylalanine and 3,3-D2 phenylalanine enrichment in muscle. At 60min after stopping the 3,3-D2 phenylalanine infusion, the final two biopsies will be obtained, again one from each leg to measure the dilution in intracellular free 3,3-D2 phenylalanine enrichment in muscle and enrichements in [ring-13C6] phenylalanine. Blood samples will be collected every hour for the first 4-hours to measure L-[ring-13C6] phenylalanine and 3,3-D2 phenylalanine enrichment in blood plasma. During the decay period, blood samples will be collected every 10 minutes to assess the decay in 3,3-D2 phenylalanine in blood (18). In total the young subjects will have 7 muscle biopsies and 13 blood samples (13 x 8 mL = 104 mL total). Note that the second blood sample in the 72-hour immobilization trial will also serve as the first blood sample in the acute FBR trial. This visit is expected to last 7 hours.

Intervention

Three days of immobilization via a full leg cast
One leg will be immobilized at a 30 degree knee joint angle of flexion for 3 days by means of leg cast (see Figure 1). The leg to be immobilized will be randomized and balanced in the study between left and right. Prophylactic medication to prevent deep vein thromboses (DVT) is not warranted when a single joint (in this study the knee joint) is immobilized (21). However, to further minimize the risk of DVT development all subjects will perform daily exercises to activate the calf muscle pump. The time needed to perform these exercises is 5 minutes. Exercises will be performed 3 times a day (i.e. morning, afternoon and evening) for the 3 days of the immobilization period. Subjects will be provided with crutches because they are not allowed to bear weight on their immobilized leg. Full instructions will be given on the correct use of crutches. Although subjects will be provided with and instructed on the use of crutches, subjects are most likely to experience a large degree of physical impairment during the 3 days of immobilization. Activities like driving a car and/or bicycle, and sport activities will be prohibited. Other activities might require assistance (e.g. travelling, climbing stairs). Walking is only permitted with crutches, without weight bearing on the immobilized leg. Overall, it is important to note that for the duration of the immobilization period physical mobility will be severely hampered for all subjects.
Following the removal of the cast, subjects are likely to experience some of the effects of a reduction in muscle mass and strength of their immobilized leg. Normal mobility and activities of daily living will no longer be impaired, but sports performance may be somewhat below normal level for 2-14 days.
Throughout the immobilization and recovery period the researcher will establish regular contact with the participating subjects to discuss any problems or queries. During the recovery period all subjects are also at liberty to seek contact with the researcher, physiotherapist and/or physician to discuss any possible problems related to the immobilization period. Given the short duration (3 days) of the recovery period, we expect muscle mass and function to restore itself entirely within a few weeks after cast removal.

Leucine
During immobilization, subjects will receive bottles containing either leucine or carbohydrate (maltodextrine) in a double-blinded manner to ingest with each main meal (breakfast, lunch, dinner). Subject will be instructed to add water to the bottle and ingest the supplement. Each supplement serving will amount to 5.0 g leucine or carbohydrate for a total of 3 bottles or 15 g per day.

Study burden and risks

The risks involved in participating in this experiment are minimal. If the subjects are interested, they will receive their own results from the tests.
The subjects will receive reimbursement for their time investment and burden of the study. Besides this, there is no additional benefit for participating in this study.
The incision made for obtaining the muscle biopsy will be performed under local

anesthetic by an experienced physician. Within our research group we have extensive experience with taking muscle biopsies. There is a small risk of infection and the muscle biopsy canlead to minor hematoma. During the blood draw there is a small risk of fainting or haematoma. These risks are minimized by using trained and experienced personnel for taking the muscle biopsies and blood draws. Adequate pressure will be applied after the blood draw and biopsies to minimize the risk of developing a hematoma.
The Aviko meals are normal food products and have been cleared for human consumption. There are no complications associated with the procedure of a single slice lower limb CT scan. The level of radiation emitted is very low; approximately 0.053 mSv per CT scan. In this study, subjects will undergo two CT scans (one from each leg both before and after the immobilization period) and will therefore be subjected to 0.106 mSV of radiation. These scans are routinely performed in studies by our group (e.g. see MEC 06-3-062, 11-3-073, 12-3-012). The DEXA scan will be used to assess body composition. Subjects will be exposed to 0.001 mSv of radiation from the DEXA scan. For comparison, the average person living in The Netherlands is exposed to ~2.5 mSv/year in background radiation. For comparison, the average person living in The Netherlands is exposed to ~2.5 mSv/year in background radiation. The MRI scan is non-invasive, though subject can feel somewhat unpleasant as the scanner will make some noise when making the images, thereby, the subjects will be lying on a table in a small *tunnel*.
In terms of time investment to visit the University, the screening visit will take 1.5 hours of the subject*s time. The baseline testing (Test Day #1) will take ~2 hours of the subject*s time. The onset of immobilization visit (Test Day #2) will take ~2 hours. Lastly, the end of immobilization visit (Test Day #3) will take ~2 hours for the older subjects and ~7 hours for the younger subjects. An additional burden will be the requirement for subjects to fill in the food diaries and wear the Actical physical activity monitor (Philips) both for 3 days before and (see appendix F2.1 and F2.2) during the immobilization intervention (6 days in total). However, the greatest inconvenience in terms of time and also mobility will be the 3 days of one-legged immobilization. One leg will be immobilized for 3 days by means of a full leg cast at a 30 degree angle of flexion (see Figure 1). In a healthy population deep vein thrombosis (DVT) will develop in 1 out every 1000 individuals a year (51). After surgery and/or bone fracture, immobilization of two or more joints will increase the risk of developing DVT. The incidence of DVT occurrence during limb immobilization following surgery and/or bone fracture is estimated between ~0.2 and ~17% (21, 52-54). However, in the present study prophylactic medication to prevent DVT is not warranted as only a single joint is immobilized (21, 52) particularly for a time period of only 3 days. However, to further minimize the risk of DVT development, subjects with any history of thrombosis will be excluded (please see exclusion criteria; section 4.3) and all subjects will perform daily exercises to activate the calf muscle pump. The time needed to perform these exercises is 5 minutes. Exercises will be performed 3 times a day (i.e. morning, afternoon and evening) for the 3 day immobilization period. Finally, throughout the immobilization period all subjects will be monitored closely by

the research team, who will establish regular contact with the subjects, to identify early signs of DVT development and act accordingly. Thus, in this study the overall risk of DVT is minimal (<0.1%). The leg cast will cause a large degree of physical impairment. Subjects will be provided with crutches as they are not allowed to bear any weight on their immobilized leg. Full instructions will be given on the correct use of crutches. To minimize the risk of any injury due to this loss of physical mobility, activities like driving a car and/or bicycle, and sport activities will be prohibited for the 3 day immobilization period. Other activities might require assistance (e.g. travelling, climbing stairs). Walking is only permitted with crutches, without weight bearing on the immobilized leg. During the first days/week after removal of the leg cast subjects may encounter the effects of the reduced muscle mass and strength of their immobilized leg. Normal mobility and activities of daily living will no longer be impaired, but sports performance might be somewhat below normal level in the first week after the study. However, since only healthy subjects will participate in this study, muscle function will most likely completely restore itself within a few weeks after cast removal (55) Indeed, it has been shown that even after 3 weeks of leg immobilization in young individuals, the resumption of spontaneous activity for 2 weeks results in the recovery of 90% of muscle strength and 95% of muscle fibre size (55). A final follow-up phone call or e-mail will be performed 6 weeks after cast removal to confirm that full recovery has taken place. If this is not the case, further follow up will be performed, subjects will receive individual advice from a sport physician and/or physiotherapist on a short recovery training program to restore muscle mass and strength, or referral to the general practitioner will take place when appropriate.
As discussed above, the leucine supplement is widely accepted for scientific and clinical purpose. No major risks are associated with the use of leucine, even for longer periods or when using higher dosages than in the current study, in healthy subjects (34, 42, 43, 47).

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

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

Inclusion criteria

* Moderately active
* Male or female age 18-35 or 60-80 years of age inclusive
* BMI not lower than 18.5 and not higher than 30 kg/m2
* Having given informed consent

Exclusion criteria

* Previous participation in a 13C amino acid tracer study (within the last 5 years)
* Lower limb and/or back injuries
* A history of thrombosis/cardiovascular disease
* Use of anticoagulants
* Musculoskeletal/orthopedic disorders
* Structured resistance exercise training
* Use of corticosteroids
* Current use of protein supplements (during the study)
* Diabetes (type I or II)
* Pregnancy
* Hormone replacement therapy
* Third generation oral contraceptives
* Use of tobacco products

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):	24-04-2016
Enrollment:	64
Type:	Actual

Ethics review

Approved WMO

Date:	30-12-2015
Application type:	First submission
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: 23203
Source: Nationaal Trial Register
Title:

In other registers

Register	ID
CCMO	NL55456.068.15
Other	Will be registered at the Dutch trial register as soon as the METC registrationnumber for this protocol is provided
OMON	NL-OMON23203