INFORMATION TO PARTICIPANTS INVOLVED IN RESEARCH

You are invited to participate

You are invited to participate in a research project entitled: **The effect of single lower limb unloading on exercise performance, fatigue and potassium regulation in healthy young adults**

This project is being conducted by Prof. Michael McKenna, Dr Suzanne Broadbent, Dr Nigel Stepto, Dr Francois Billaut and Dr Aaron Petersen, Dr. Itamar Levinger and Mr. Ben Perry.

Project explanation

This research is being conducted to assess what effect inactivity, a huge problem in public health, has on various markers of muscle physiology and general health. This research also seeks to investigate whether electrical muscle stimulation in humans can help attenuate some of the effects normally seen with inactivity. This project aims to investigate the effects of 23 days of single lower limb inactivity on exercise performance, muscle physiology and biomedical health markers. This research will also investigate whether electrical muscle stimulation can minimise the effect of inactivity on a single leg.

What will I be asked to do?

We will ask you to fill in several short questionnaires about your family medical history and your exercise habits.You will then be asked to do a familiarisation of the exercise tests, using crutches, and a maximal aerobic test ($VO_{2\ peak}$). You will then start the formal exercise tests which span over two visits (total of six visits for exercise testing), one of which will involve blood sampling, muscle samples (from the thigh) and peripheral magnetic stimulation. All muscle samples will be performed by a qualified medical practitioner. This process, called a *muscle biopsy* is performed a under local anaesthetic, and involves a small sample (about 3-4 grains of rice) of muscle to be taken. After three days rest, you will be asked to do daily activities with the use of crutches, and a raised-sole on one shoe. This will cause one leg to not undertake activity (similar to if you were to injure a knee or ankle and you required crutches). If you agree to the use of crutches and the raised shoe, you will be asked to use crutches for 23 days, in this time you will report in to the exercise physiology laboratory (LCESS building, Victoria University, Footscray park campus) twice per week for blood testing. If you are in the group to receive muscle electrical stimulation, you will be asked to participate five times per week in a training protocol utilising muscle electrical stimulation during the time in crutches. After the 23 days, you will then be asked to complete the same exercise testing, with blood and muscle samples, as completed previously. You will then be asked to undertake a strength training program for the next four weeks (three times per week). This program is designed to return your strength power and muscle size to that or exceeding that acquired before participating in the study. You will then be asked to do the final round of exercise testing (the same tests as previously completed). A further 3 weeks of resistance training will be offered to you if you wish to keep training.

During the study, you will also be asked to have a DXA scan (Dual X-ray absorptiometry) on your lower limbs. This will occur three times during the study, and you will be asked to meet one of the investigators at Victoria University (Footscray Park) to complete this test. This test will be used to accurately measure lean muscle mass in the legs. The DXA scans involved in this research study involves exposure to a very small amount of radiation. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2 millisievert (mSv) each year. The effective dose from this study is about 0.03 mSv. At this dose level, no harmful effects of radiation have been demonstrated as any effect is too small to measure. The risk is believed to be minimal. Additionally, your blood samples will be measured for basic metabolic health markers (Glucose, triglycerides and cholesterol). Weight, height, blood pressure and waist circumference will also be required throughout the study

What will I gain from participating?

You will gain a thorough, scientific insight into multiple aspects of your fitness. You will gain a highly accurate measure of your muscle and fat mass. You will receive free training with supervision at Victoria rehabilitation clinic for up to seven weeks, and will be shown various resistance exercises for the lower limbs. Additionally, you will be payed an honorarium of $400 at the completion of your participation, and will gain the knowledge of your muscle fibre distribution; something which can only be attained from studies such as these. Finally, you would be contributing to knowledge in an area which is becoming more prevalent, the effects of inactivity in humans.

How will the information I give be used?

The information which will be gained from the research will be used for analysis of various muscle markers of muscle excitability and fatigue (Na^+/K^+ pump activity, content and expression) and how they are altered after inactivity, and whether electrical stimulation alters this. Additionally, single fibre characteristics will also be determined. Strength and muscle cross sectional area data will be used to assess whether the ULLS protocol was effective, and if electrical stimulation causes a functional adaptation during inactivity. Your samples and data will be stored under alphanumeric codes (i.e. without your name or personal details) and only the researchers will be able to connect the samples to you. The data that will be collected during the study will be used/published in peer-reviewed journals and conference presentations. No personal details will be revealed without your written consent.

What are the potential risks of participating in this project?

The maximal cycle ergometer incremental exercise test and one legged incremental cycling test involve the risk of sudden death due to myocardial infarct, vasovagal episodes, muscle soreness and stiffness. Risks associated with venous catheterisation include discomfort, bruising and infection (for example puss, tenderness and/or redness). Risks associated with muscle biopsy include discomfort, pain, bruising, bleeding, soreness, localised altered sensation of skin reduced /absent /tingle /hypersensitive) and infection. Risks associated with being on crutches for 23 days include increased risk of deep vein thrombosis, decreased mobility and independence, increased risk of falls due to the use of crutches, decreased muscle strength, atrophy, muscle cramping and muscle soreness. A regular blood sample will be taken while on crutches to test for a pre-clinical marker of deep vein thrombosis (DVT). Symptoms of DVT include redness, swelling and pain in the affected leg. Aspirin (100 mg) will be

supplied to you for daily consumption as a preventative measure against DVT. ULLS does increase the risk of DVT or a superficial vein thrombosis, to an incidence of ~1-2%. In the incident of a possible DVT or superficial vein thrombosis forming, you will immediately be referred to a medical doctor for a Doppler ultrasound, with any costs for treatment being covered by funding from the research. You should continue to check for the symptoms of DVT (redness swelling and/or pain in the leg) daily after the ULLS protocol for up to one month. Thereafter, continue to regularly check for the signs and symptoms of DVT for an additional two months after the ULLS protocol. If any signs and symptoms of DVT are noted, immediately seek medical consultation and contact the researchers. Psychological risks from the ULLS protocol include frustration and anxiety from lack of mobility, and frustration from being unable to undertake exercise for the 23 day period.

Inclusion criteria:

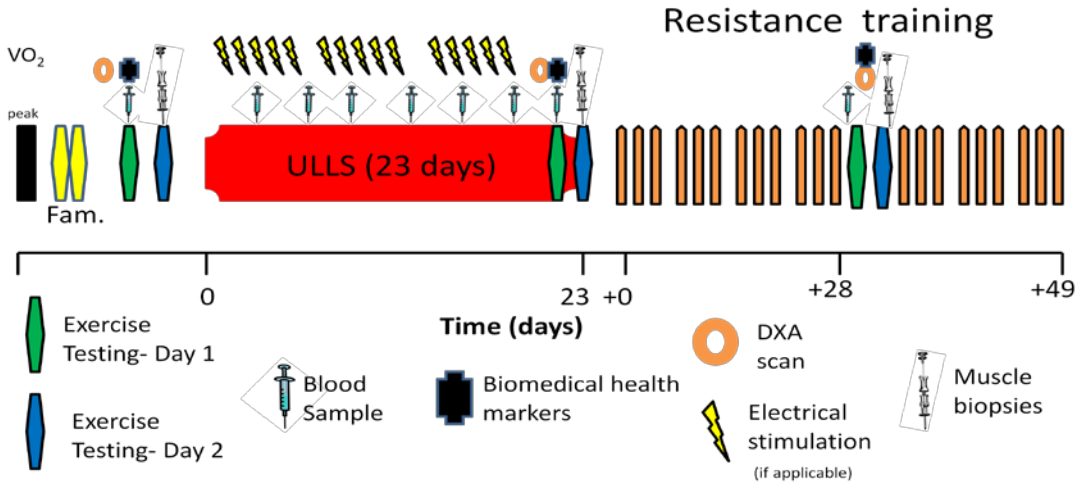
- Between the age of 18-35 years of age.
- In good health.

Exclusion criteria:

- Using oral contraceptives.
- A family history of DVT
- Blood clotting problems/disorder
- Any allergies to aspirin
- Obesity.

How will this project be conducted?

As a potential participant you will be initially screened for cardiovascular risk factors and any health issues of relevance to the study. If you are deemed healthy and at low risk of any adverse events you will be ask to complete multiple testing sessions as listed below. The study involves multiple visits to VU, Footscray Park campus over a total 9-12 week period. The schematic on the next page demonstrates the required involvement in the study. This research is completely voluntary; you are free to withdraw from the study at anytime without resentment or ramifications from any of the investigators. However, withdrawal after more than a week of ULLS will leave you with a slightly weaker leg. The testing of DVT will still be offered to you after the inactivity period even if you withdraw at anytime during ULLS.



Visit 1 (~60min): Body Composition, Aerobic fitness and Familiarisation to crutches.
During this session you will undertake some basic measures of body composition including determination of height, weight and waist circumference. Immediately following these measures you will then undertake an aerobic fitness (VO_{2peak}) test by performing an incremental exercise test on a cycle ergometer. After recovery, you will be asked to briefly (~5 minutes) ambulate on crutches with an extended show. This is to let you know how it will feel if you have never been on crutches before.

Visit 2 (~2h): Familiarisation Day 1
This will occur approximately one week after your $VO_{2\ peak}$ test. During this session you will perform familiarisations of the exercise tests to be performed. These include proprioception (balance) tests, vertical jump and quadriceps strength test. Data will not be collected; this session is to ensure you are comfortable and competent with the exercise testing. You will be given substantial breaks between each test to ensure there is no fatigue before the exercise begins.

Visit 3 (~3h): Familiarisation Day 2
This visit will occur approximately 1-3 days after your last visit. Like the last visit, this session is for your familiarisation to the exercise testing. This session will include the one legged incremental cycling protocol (on both legs), with a ~2 hour break between each leg tested. You are asked to wear comfortable exercise gear in all exercise testing.

Visit 4 (<1h): DXA scan
You will attend the Western Hospital (Footscray), or Sunshine Hospital, St. Albans with one of the investigators for a DXA scan. This whole body scan calculates muscle, bone and fat proportions. The scan uses very low levels of ionising radiation; it utilises less than 1/10 of the radiation emitted from a chest x-ray.

Visit 5 (~2.5h): Baseline testing Day 1
This testing will occur approximately 3-7 days after your familiarisation. This testing day will consist of the following exercise tests: Vertical jump, quadriceps strength and lower limb proprioception. This is the same tests which were performed on visit 2. You will be given appropriate rest between each test to ensure that there is no fatigue at the beginning of each test.

Visit 6 (~4h): Baseline testing Day 2 (One legged cycling protocol)
This exercise testing session will occur the day after the last visit. This is the same exercise test as familiarised as on familiarisation day 2, but this test will also involve blood sampling from venous cannulation, magnetic stimulation (causes muscle contraction), Electromyographic recording of muscle activity and muscle biopsies from the thigh. Additionally, a mask will be fitted to measure expire gases as was used in the first visit ($VO_{2\ peak}$). A total of two muscle biopsies will be taken: one on each leg at rest

Visit 7-13 (<1h): Unilateral Lower Limb Suspension (ULLS), blood tests and familiarisation.
Three days after the second day of baseline testing, you will be issued with crutches and a customised shoe which will be required to be used for the next 23 days. You will be familiarised with ambulating on crutches, and it will be ensured the equipment supplied is adjusted correctly for your height. You will also be given a compression garment for the inactive leg, and a small pressure pad for the insert of the shoe. The pressure gauge will only monitor the amount of weight placed on the non-weight bearing leg; it cannot retain

any information on the destination of the unit. Additionally, if you are selected to receive electrical stimulation, you will be familiarised with the training protocol.

Throughout the 23 days on crutches and the raised sole shoe, you will be required to attend the exercise physiology laboratory twice per week (five times if assigned to electrical stimulation training group) for a single blood sample via venepuncture. This blood sample will test whether there is an increased risk of DVT development. The first and final blood samples will also be used for fasting glucose, triglycerides and cholesterol. Additionally, this is an opportunity for you to discuss any problems/issues you are having with the crutches/study in person during this time. You will also be supplied with the contact details of one of the investigators throughout the study if any issues arise.

Additionally, you may be randomly selected to receive electrical muscle stimulation five times per week while under ULLS. This electrical stimulation training is brief (<30 minutes), and muscle stimulation intensity is kept at a safe and painless level attained during the familiarisation before the ULLS period.

Visit 14 (<1h): DXA scan
Same as previously described DXA scan. Will occur one day prior to the end of ambulating on crutches (day 22)

Visit 15 (~2.5h): Post ULLS testing day 1
This testing day will occur on day 21 of being on crutches. This will be the same tests as the first day of baseline testing. You will undertake the vertical jump, quadriceps strength and proprioception tests.

Visit 14 (~4h): Post ULLS testing day 2
This will occur the day after the last testing session. This will be the same exercise testing protocol (with blood samples and muscle biopsies) as used on baseline testing day 2; the one legged incremental cycling test is completed on both legs. The only difference in this exercise test compared to the previous test is that there are two additional muscle biopsies taken, each after exercise on the cycling leg.

Visit 15-27 (<45 min): Strength training program at Victoria University
3-5 days after the exercise testing, you will begin a strength training program at the Victoria University rehabilitation clinic (Footscray Park) under the supervision of a Postgraduate student in exercise rehabilitation. The program will last for four weeks, with three training programs per week (total 12 sessions). The training will focus primarily on the lower limb affected while you were on crutches. The sessions will last 30-45 minutes, and will be held at a time convenient for your schedule.

Visit 28 (~1.5h): Post resistance training testing Day 1
After four weeks of strength training, you will be asked to complete the final bout of exercise testing. The schedule will be the same as previously described with day 1 consisting of the vertical jump test, quadriceps strength and proprioception testing.

Visit 29 (~4h): Post resistance training testing Day 2
Two days after the previous testing session, you will be required to complete the one legged incremental cycling test using the same protocol, with blood samples and muscle biopsies, as previously described. This test is identical to the first occasion (before ULLS) the one-legged cycling test was completed, there is a total of two muscle biopsies at rest; one on each leg.

Visit 30 (<1 h): DXA scan
Same as previously described DXA scan.

Who is conducting the study?

The study is conducted by the School of Sport and Exercise Science, Victoria University

Main Investigators:

Prof. Michael J. Mckenna, Telephone number 9919 4499, Mobile 0488 475 735,
Email: michael.mckenna@vu.edu.au

Dr. Nigel K Stepto, Telephone number 9919 5416, Mobile 0409 338 696,
Email: nigel.stepto@vu.edu.au

Dr. Aaron Petersen Telephone number 9919 9452 Mobile
Email: Aaron Petersen@vu.edu.au

Dr. Francois Billaut Telephone number 9919 9451 Mobile
Email: Francois.Billaut@vu.edu.au

Dr. Suzanne Broadbent Telephone number 9919 5981 Mobile
Email: Suzanne.broadbent@vu.edu.au

Prof. Graham Lamb Telephone number 9479 2249
Email : G.Lamb@latrobe.edu.au

Dr. Robyn Murphy Telephone number 9479 2302
Email : R.Murphy@latrobe.edu.au

Dr. Cedric Lamboley Telephone number 9919 4287
Email : cedric.lamboley@vu.edu.au

Dr. Itamar Levinger Telephone number 9919 5343
Email : itamar.levinger@vu.edu.au

Mr. Ben Perry Telephone number 9919 4207 Mobile 0414600986,
Email: ben.perry@live.vu.edu.au

Any queries about your participation in this project may be directed to the Principal Researcher listed above.
If you have any queries or complaints about the way you have been treated, you may contact the Secretary, Victoria University Human Research Ethics Committee, Victoria University, PO Box 14428, Melbourne, VIC, 8001 phone (03) 9919 4781.