EXERCISE METABOLISM RESEARCH GROUP
DEPARTMENT OF KINESIOLOGY, MCMASTER UNIVERSITY

PARTICIPANT INFORMATION SHEET AND CONSENT FORM

Effect of exercise on the human skeletal muscle phosphoproteome

Funding Source: Canadian Institutes of Health Research**,** National Institutes of Health

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You are being invited to participate in a research study looking at the effects of resistance exercise (i.e., weight lifting) and endurance exercise (i.e., cycling) on skeletal muscle adaptations to exercise. In order to decide whether or not you want to be a part of this research study, you should understand what is involved and the potential risks and benefits. This form gives detailed information about the research study, which will be discussed with you. Once you understand the study, you will be asked to sign this form if you wish to participate. Please take your time to make your decision. Feel free to discuss it with your friends and family. McMaster University and Professor Stuart Phillips, as well as Dr. Troy Hornberger (University of Wisconsin-Madison) have received grants from the Canadian Institutes of Health Research (CIHR), and the National Institutes of Health (NIH), respectively, to cover the costs of conducting the study.

PURPOSE OF THE STUDY

Skeletal muscle adapts rapidly and robustly to exercise. The adaptations induced by regular training are diverse and depend primarily on the nature of the exercise stimulus. Generally, resistance exercise increases muscle mass and strength, whereas endurance exercise increases the capacity to use fuels (i.e., to "burn" sugar and fat) and fatigue resistance. In both cases, skeletal muscle translates the physical and biochemical stresses of exercise into morphological and metabolic adaptations, with the adaptations improving one's ability to perform the corresponding type of exercise. This concept is known as training specificity, and although it makes intuitive sense, the molecular mechanisms that link resistance and endurance exercises with their specific adaptations are not fully understood. How resistance exercise achieves these adaptations remains understudied, but what is known is that skeletal muscle translates the physical and biochemical stresses of resistance exercise into morphological and metabolic adaptations. While both types of exercise activates signaling pathways (i.e., proteins) that increase the synthesis of specific proteins to cause adaptations, thousands of proteins are likely involved, and their interactions are complicated. Until recently, our analytical capacity has limited the number of pathways that could be studied simultaneously; however, using novel molecular techniques, we are examining the entire complement of skeletal muscle proteins in response to resistance exercise. By examining all of the proteins in skeletal muscle, we will be able to indentify those proteins that mediate resistance and endurance exercise adaptations. Specifically, our aims are to identify the unique and overlapping pathways activated by these distinct types of exercise and to assess

changes in the rates of protein synthesis that occur in response to each. Our goal is to better understand how exercise training improves the capacity of muscle to perform exercise.

INCLUSION AND EXCLUSION REQUIREMENTS

How many people will be recruited for the study?

If you consent, you will be one of 12 participants included in this study.

Inclusion Requirements

In order to participate in this study, participants must be male or female, between the ages of 18 and 30 years, and have performed a mix of resistance exercise and endurance exercise 3 times per week for at least 1 year

Exclusion Requirements

Participants cannot participate in or will be released from this study if they meet any one of the following criteria:

- Smoker or user of tobacco products;
- High physical activity;
- Have health problems such as: renal or gastrointestinal disorders, metabolic disease, heart disease, vascular disease, rheumatoid arthritis, diabetes, poor lung function, uncontrolled blood pressure, dizziness, thyroid problems, or any other health conditions for which you are being treated that might put you at risk for this study;
- Taking anti-diabetic, anti-inflammatory, platelet inhibitor, or anti-coagulant medications;
- Use of an investigational drug product within the last 30 days;
- Have participated in an infusion protocol in the last year; or
- Do not understand English or have a condition the PI believes would interfere with a participants' ability to provide informed consent, comply with the study protocol, or which might confound the interpretation of the study results or put someone at undue risk.

PROCEDURES INVOLVED IN THE RESEARCH

Time Commitment

The complete time commitment of this study will be a period of approximately 3 weeks. Your first meeting will include a participant-screening interview during which the researchers will determine if there are any reasons that you cannot take part in the study. Upon your consent, we will determine a start date for the study. Throughout the study you will visit McMaster University on at least six occasions (see attached study timeline).

Initial meeting

You will come to McMaster University to meet with our study coordinators (Dr. Chris McGlory and Mr. Jonathan Mcleod) who will explain the study protocol. Should you wish to participate, you will then provide informed consent. Following this meeting, we will schedule your future visits to undergo baseline testing and experimental procedures, which include a body

composition scan, resistance exercise testing and familiarization, and muscle biopsies and an infusion in conjunction with exercise.

Study Visits

Visit 1
During the first visit, you will arrive at McMaster University following an overnight fast (approximately 10 hours). First, you will undergo a body composition scan, using a dual-energy x-ray absorptiometry (DXA) machine. Afterwards, you will be familiarized with the resistance exercise and endurance exercise equipment that will be used on future visits. At this point, your two legs will be randomly assigned to the resistance exercise or endurance exercise group.

Visit 2
On the second visit, you will undergo a single-leg resistance exercise test. This test will determine the maximum amount of weight that your one leg can lift on a leg press and leg-extension machine. This test will determine the load lifted during the experimental trials.

Visit 3
On the third visit, you will undergo a VO2max test (i.e., an endurance test) to determine the maximum amount of oxygen taken up by your body. This test will be performed with two legs on a stationary bicycle. This test will provide an indication of your aerobic fitness. Following the test, you will be familiarized with single-leg cycling.

Visit 4
On the fourth visit, you will undergo a single-leg VO2max test to determine the maximum amount of oxygen taken up by your body when only one leg is exercising. This test will be performed on a stationary bicycle adapted for one-leg cycling, and it will determine the intensity of your endurance exercise during the experimental trials. Following this test, and after a brief rest, you will complete a familiarization trial of the single-leg cycling protocol with your opposite leg.

Visits 5 and 6
On your fifth visit to the laboratory, you will complete the first experimental trial. For the three days prior to this visits, you will be provided with a standardized diet. On the day of the trial, you will arrive to the laboratory after an overnight fast (~10 hour), at which point we will measure your height and weight. After resting for three hours, you will undergo a muscle biopsy and then perform the two single-leg exercise protocols. The **resistance exercise protocol** consists of 3 sets of 10 reps for leg press and leg extension, performed at 70% of your 1-repitition maximums (determined on visit 2). The resistance exercise will take approximately 20 minutes and will be followed by a muscle biopsy. After that protocol is completed, your opposite leg will perform the **endurance exercise protocol**, which consists of a 5-min warm-up and four 5-min intervals that are interspersed with 2.5-min recovery periods. The endurance exercise protocol will take 35 minutes and will be followed by a muscle biopsy. You will then rest for the remainder of the trial, with muscle biopsies collected 3 hours after the cessation of each exercise protocol.

Visit 6 will be identical to visit 5, except that, on arrival, a catheter will be placed into a vein in your arm to allow for the infusion of a stable amino acid isotope. This infusion will continue for the remainder of the that day's trial. A catheter will be placed into the opposite arm to allow blood sampling throughout the experimental trial.
The muscle biopsy and catheter placement will be performed by highly trained personnel under the assumed medical responsibility of the overseeing physician Dr. Steven Baker.

Study Measures

Dual-Energy X-Ray Absorptiometry (DXA) Scan

A DXA scan will be used to determine your muscle size and body composition (see risks below). The DXA procedure uses a small amount of radiation to determine how much fat, bone, and muscle mass you have in your entire body. This procedure takes approximately seven minutes and involves lying still on an open bed while the sensor passes over the body.

Single-leg one-repetition maximum

You will be instructed on the proper way to perform single-leg extensions using a leg-extension machine. The same investigators will administer all strength testing. In short, after a brief general warm-up, a specific warm-up of the given exercise at approximately 50% of your estimated one-repetition maximum. The load will then be progressively increased by approximately 10-20% for each repetition until a true maximum repetition is reached. Three to 5 min of rest will be given between each attempt.

Maximum oxygen uptake test (VO2max)

This test involves cycling on a stationary bike at progressively higher workloads while the amount of oxygen taken up by your body is determined from a facemask connected to a gas analyzer. This test will also determine your maximal workload on the cycle ergometer, which is used to assess your fitness and determine the intensity for the experimental protocol. We will also measure your heart rate during this test. This test will be completed with two legs (i.e. conventional cycling) and with a single leg.

Muscle Biopsies

During the study, you will have **10** muscle (five each on Visits 5 and 6). Professor Stuart Phillips will be performing the muscle biopsies, under the medical supervision of Dr. Steven Baker. He has performed over 6000 biopsies in young and elderly adult subjects. This procedure involves the removal of a small piece of muscle tissue using a sterile hollow needle. He will clean an area over your quadriceps muscle (*vastus lateralis*) and inject a small amount of local anesthetic ("freezing") into and under the skin. He will then make a small incision (approximately 4-5 mm) in the skin in order to create an opening through which to put the biopsy needle into your thigh. He will then quickly cut off a very small piece of muscle (approximately 50-100 mg; about the size of half an eraser on the end of a pencil) and remove the needle from your leg. Following the

biopsies, the incisions will be closed with instant medical adhesive, and wrapped with a tensor bandage.

Stable isotopes

These stable isotopes are not radioactive, are made under sterile conditions, and are passed through a very selective filter before entering your body. These isotopes are already found naturally in your body and are not harmful to you.The stable isotope is dissolved in a salt solution similar to your blood and poses no health risks since it is not radioactive. It will simply result in an increase in the level of stable isotope within your body for a short period of time (months). All of the infused solutions are prepared under sterile conditions and passed through a very selective filter before entering your body.

POTENTIAL HARMS, RISKS OR DISCOMFORTS

DXA scan

The radiation dose from one DXA scan is approximately 10 micro Sieverts, 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.). This procedure is painless and non-invasive.

Venous Blood Sampling and Infusion

The insertion of a needle for blood sampling is a common medical practice and involves minimal risk provided proper precautions are taken. The needle is inserted under sterile conditions; however, there is a theoretical risk of infection. There is also a chance of bleeding if enough pressure is not put on the insertion site when the needle is removed. This may cause some minor discomfort and could result in bruising/skin discoloration that could last up to a few weeks. There is also a small risk that damage 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 these types of problems in our laboratory after several thousand venous blood-sampling procedures.

Muscle Biopsy Procedure

All biopsies will be performed by fully trained and qualified personnel. The biopsy technique is routinely used in research and complications are rare provided that proper precautions are taken. During the time that the sample is being taken (approximately 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. You should not do strenuous exercise for the rest of the day. Once the anesthetic 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 Acetaminophen (Tylenol) or Ibuprofen (Motrin) can be taken if you experience pain associated with the biopsy. It is also beneficial to periodically apply an ice

pack to the biopsy site the following day, 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.

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. 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, but baths, swimming, saunas, etc. should be avoided for at least 4 days following the biopsy procedure. 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 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 small 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, 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 approximately 2-3 weeks by rubbing the area); 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 approximately 4-5 days. To the best of our knowledge, older subjects who have undergone the muscle biopsy procedure have reported no adverse reactions. While there is also a theoretical risk of damage to a small motor nerve branch (these allow your muscle to move) of the *medial vastus lateralis* muscle, this has never been seen in over 9,500 biopsies performed by the investigators at McMaster University and the risk of damaging a small motor nerve branch is considered small.

Exercise testing

The potential risks and discomforts associated with the exercise testing procedures are similar to those associated with any form of physical activity. These include fatigue, fainting, abnormal blood pressure, irregular heart rhythm, and in very rare instances, heart attack, stroke or death. Every effort will be made to minimize these potential risks by evaluation of preliminary information relating to your health and fitness and by careful observations during testing.

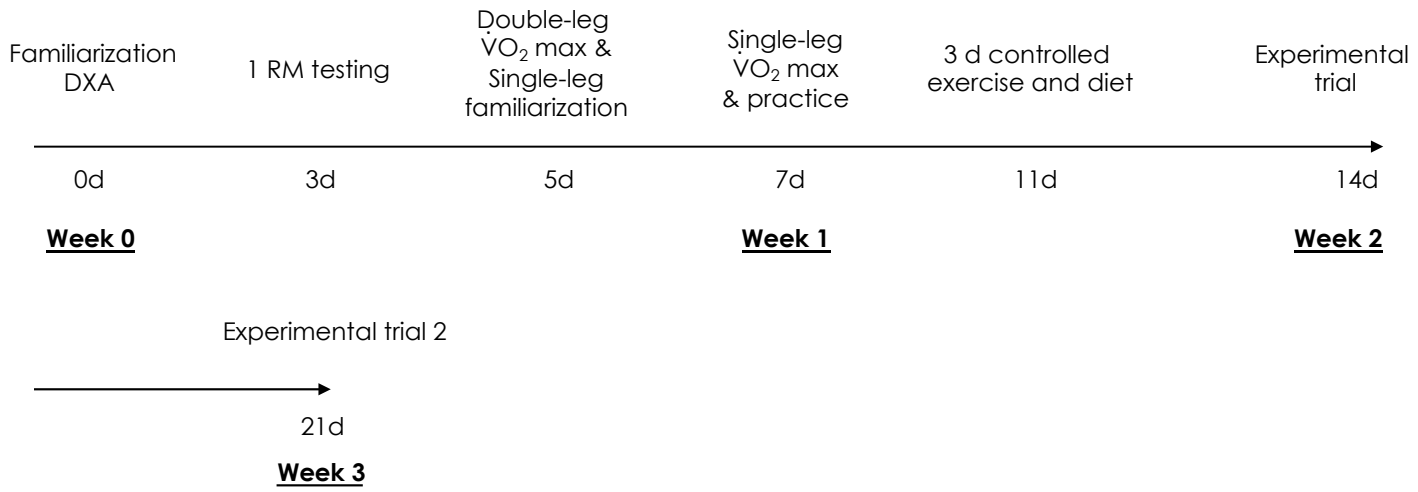

Figure 1. **The timeline of the study procedures**. DXA, *Dual Energy X-Ray Absorptiometry (DXA) Scan; 1-RM, one-repetition maximum; VO2max, maximum oxygen uptake. Note that the "3 d controlled exercise and diet" is not a visit to the laboratory; it is a period in which we will provide you with all of your food and you will not be permitted to perform exercise.*

RESEARCH RELATED INJURY

If you are injured as a direct result of taking part in this study, all necessary medical treatment will be made available to you at no cost. Financial compensation for such things as lost wages, disability or discomfort due to this type of injury is not routinely available. However, if you sign this consent form it does not mean that you waive any legal rights you may have under the law, nor does it mean that you are releasing the investigator(s), institution(s) and/or sponsor(s) from their legal and professional responsibilities.

POTENTIAL BENEFITS

There are no proposed benefits to you as the subject of this study.

PAYMENT OR REMUNERATION

In participating in this study, you realize that there are no direct benefits to you. You will receive an honorarium of $250 upon the completion of the study to compensate you for your time commitment. Should you wish/be required to withdraw from the study at any point, we will reimburse you on a pro rata basis for the time you have spent on the study (i.e. half of the study completed = $125).

PLEASE NOTE: Remuneration will be in the form of a cheque issued by McMaster University. The cheque will be mailed to you at the completion of the study for the full amount. While we will attempt to have the remuneration forwarded to you as soon as possible, it may take up to four weeks following the completion of the study for the cheque to be processed and the remuneration received.

In addition to the remuneration, we will also cover your on-campus parking.

CONFIDENTIALITY

Muscle samples will be stored for up to 5 years after completion of the study for analyses. All data collected during this study will remain confidential and stored in offices and on computers to which only the investigators have access. Any identifying information will be coded, with corresponding code-identifiers to be kept stored in a locked filing cabinet under the supervision of Drs. Stuart Phillips, Chris McGlory, and Martin MacInnis. Information kept on a computer will be protected by a password. Once the study is complete, an archive of the data, without identifying information, will be deposited. All data will be stored in the locked filing cabinet under the supervision of Professor Stuart Phillips. Professor Phillips and Dr. McGlory will have access to the data and will supervise access to other researchers within the group. Information about you will not be released to any other person for any reason. If the results of the study are published, your identity will remain confidential. The data for this research study will be retained for 10 years.
For the purposes of ensuring the proper monitoring of the research study, it is possible that a member of the Hamilton Integrated Research Ethics Board (HIREB) may consult your research data. However, no records which identify you by name or initials will be allowed to leave the university/hospital. By signing this consent form you authorize such access.

You should be aware that the results of this study will be made available to the scientific community through publication in a scientific journal, although neither your name nor any reference to you will be used in compiling or publishing these results. Upon completion of the study, we are able to provide a personalized report of our findings at your request, as well as direct you towards all publications resulting from this study.

PARTICIPATION AND WITHDRAWAL

At any time you can choose whether to participate in this study or not. You may exercise the option of removing yourself or your data from the study at any time if you wish. You may also refuse to answer any questions posed to you during the study and still remain a subject in the study. If you wish to withdraw you can in writing or verbally inform any of the investigators that you no longer wish to take part. You will be thanked for your time and paid pro rata. The investigators reserve the right to withdraw you from the study if they believe that circumstances have arisen that warrant doing so.

You will receive a completed (i.e. signed) copy of this ethics form. You may withdraw your consent to participate in this study at any time, and you may also discontinue participation at any time without penalty. In signing this consent form or in participating in this study you are not waiving any legal claims or remedies.

QUESTIONS ABOUT THE STUDY

For any questions related to the study, please contact Dr. Chris McGlory at 905-921-3388. If you have concerns about your health or any of the procedures occurring during the study please contact Dr. Chris McGlory.

In the event of an emergency please contact Dr. Stuart Phillips at 905-525-9140 ext. 24465 (office) or Dr. Chris McGlory at 905-921-3388 (emergency). As with any medical emergency, you should proceed to the urgent care/emergency department of the closest hospital if an emergency situation should arise during the course of the study.

This study has been reviewed by the Hamilton Integrated Research Ethics Board (HIREB). The HIREB is responsible for ensuring that participants are informed of the risks associated with the research, and that participants are free to decide if participation is right for them. If you have any questions about your rights as a research participant, please call the Office of the Chair, HIREB at 905.521.2100 x 42013.

CONSENT STATEMENT

Signature of research participant

I have read the preceding information thoroughly. I have had the opportunity to ask questions, and all of my questions have been answered to my satisfaction. I agree to participate in this study. I understand that I will receive a signed copy of this form.

_____
Name of Participant

_____ _____
Signature of Participant Date (mm/dd/yy)

Consent form administered and explained in person by:

_____
Name and title

_____ _____
Signature Date (mm/dd/yy)

Signature of investigator

In my judgment, the participant is voluntarily and knowingly giving informed consent and possesses the legal capacity to give informed consent to participate in this study.

_____ _____
Signature of Investigator Date (mm/dd/yy)