PARTICIPANT INFORMATION SHEET

Title of Project: The impact of exercise intensity on vascular function in healthy young men and women.

Name of Researchers and School/Faculty: Andrea Tryfonos, Dr Ellen Dawson, Dr Matthew Cocks, Prof Daniel Green, School of Sport and Exercise Sciences

You are being invited to take part in a research study. However, before you give consent to participate in this study, it is important that you completely understand why the research is being done and what it involves. Please take time to read the following information sheet. If you have any questions, feel free to contact the researchers who will be happy to provide this information for you.

1. What is the purpose of the study?

Cardiovascular disease is the largest cause of death in the western world. Blood vessels with poor function are associated with increased risk of developing cardiovascular diseases, whereas healthy blood vessels may prevent those events. The function of a blood vessel is commonly measured using a non-invasive technique named flow mediated dilatation (FMD).

Exercise training has been demonstrated to improve blood vessels function. However, while the long-term effects of exercise training in regards to improved blood vessels function are well studied, the acute response to a single bout of exercise remains a controversial issue. Different exercise intensities are likely to result in different blood vessel function responses. Specifically, it is proposed that high intensity exercise may cause increase of factors within the blood which can change blood vessel function. This can include oxidative stress oxidative stress (an imbalance of free radicals and the body's ability to counteract their effects) and platelet function (clotting factors in the blood which are linked to cardiovascular health).

The majority of studies that examine exercise and vascular responses do not include females. Whilst sex difference plays a significant role in cardiovascular risk factors prevalence there is still a paucity of data examining the role of oestrogen to blood vessels function in response to exercise. The menstrual circle is been consisted of two phases with low and high oestrogen concentrations respectively, which may be affect differently the vascular outcomes to exercise.

Therefore, this study aims to answer several vital questions in regards to acute effects of exercise in healthy young women and men:

- Determine the time-course of FMD in response to both a single bout of exercise moderate intensity continuous (MICE) and high intensity interval exercise (HIIE).
- Investigate if there is a correlation between FMD, oxidative stress and platelet function.
- Examine if there is a sex difference in vascular effects of acute exercise and whatever menstrual cycle phase influences the acute vascular response to exercise.

2. Do I have to take part?

No. It is entirely up to you to decide whether or not to take part. If you do, then you will be asked to complete a simple medical questionnaire to assess whether you are suitable for the study, and an informed consent form. You are free to withdraw from the study at any point without giving a reason and your status will not be affected should you wish to take part in any future studies.

3. What will happen to me if I take part?

If you decide to participate in this study, you will first complete a consent form before the first measurement starts. A full explanation of all the equipment that will be used will be given and if you have any questions then please feel free to ask. It is important to us that you are completely comfortable with all of the procedures before participating. Before participation, your health status will be checked using a simple medical questionnaire.

You are **NOT** able to take part in this specific study if:

- You are more than >35 years old
- You have a history of cardiovascular disease/renal disease/respiratory disease/liver disease
- Hypertension/ hypercholesterolemia/ diabetes type I/II
- You are a smoker
- Currently taking hormone-based therapy (e.g. contraceptive pills etc.)
- Have any current injury which prevents you from exercising

No information from this screening will be shared and the personal data will be securely stored and destroyed after the study has finished.

You will then be invited to attend the **cardiovascular lab of Tom Reilly Building at Liverpool John Moores University** for the visits. A different number of visits is required for male versus female participants. Specifically, **men need to come on 5 separate visits, while women require 9 visits.**

Overall Study design: The first visit will be familiarisation and VO_{2max} test (short incremental fitness test), where you have to avoid having a heavy meal at least 3 hours and strenuous exercise 24 hours prior this session.

During the following visits (2 for males, 4 for females), you will be randomly assigned into either high intensity interval exercise (HIIE) or moderate intensity continuous exercise (MICE). You will then rest for 3-7 days and come again for the other exercise protocol.

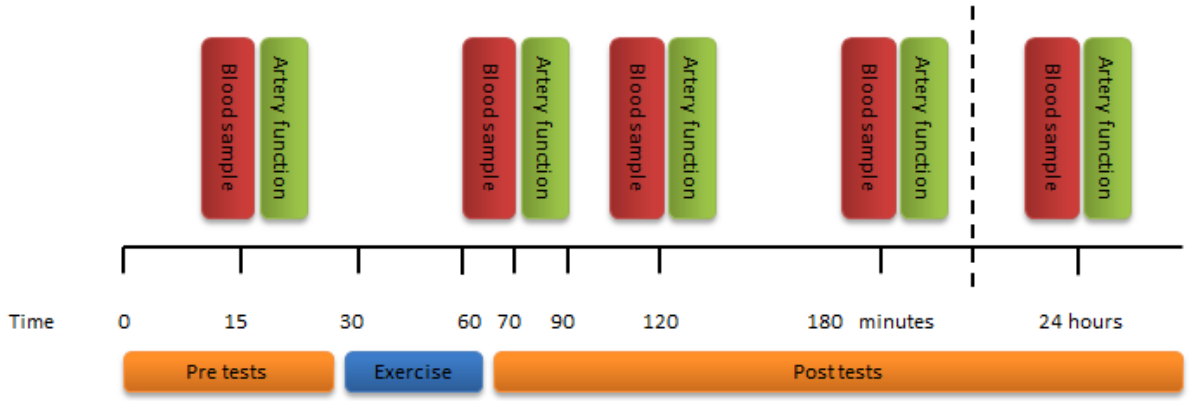
For females only: You should repeat the pattern in two different phases of your menstrual circle, low and high concentration of oestrogen. Specifically MICE and HIIE should be undertaken within both the follicular or proliferative phase of your menstrual circle (day 1-14), which is characterised by high concentration of oestrogen and the luteal or secretory phase (day 14-28), low concentration of oestrogen respectively.

Blood samples and artery function of your arm (see further explanations) will be assessed 4 times (before, and 10 min, 1h, 2h post exercise) during exercise visits (2 exercise visits for males/4 for females). You should return to the lab 24 hours after, where vascular function (1 time) and blood sample (1 time) will be taken again (2 post exercise visits for males, 4 for females).

You will be required to abstain from alcohol, vitamins, drugs and exercise for 24 hours prior to testing, and have no caffeine for 6 hours before those visits. You should have standardised breakfast (plain

toast or cereal with milk) 3 hours before those visits (keep the same breakfast for all visits expect the first one).

Figure of study protocol and time-line:



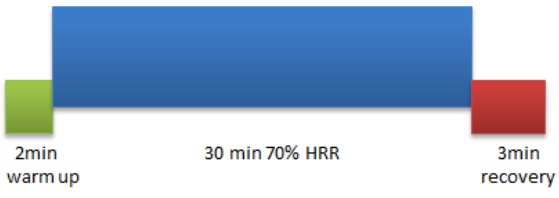
Visit 1 (familiarisation, VO_{2max} test): You will be familiarised with the equipment and the two different exercise protocols will be explained for the next visits. A simple medical questionnaire and height and weight will be obtained. You will then asked to undertake a VO_{2max} test, a short fitness test that involves cycling thought increasing levels of resistance on a stationary bicycle (resistance will be increased every 3 minutes, starting from a low resistance). During the test you will wear a heart rate monitor, and breath samples will be measured to determinate the volume of oxygen consumed. The test should be lasted 10-15 minutes, and the total duration of this visit will be approximately 30 minutes.

MICE visit (1 for males, 2 for females): You should undertake a single bout of moderate intensity continuous exercise on a stationary bicycle. The Intensity will be 70% of your maximal heart rate based on the maximal value obtained in your fitness test (VO_{2max} test). The actual exercise protocol will be last 30 minutes, with a 2min warm up and a 3min recovery in a low resistance (20 watts). In total the exercise will last for 35 minutes. Artery function of your arm and blood samples will be taken before, 10 min, 1h and 2h post exercise. This whole visit will last approximately 3 hours.

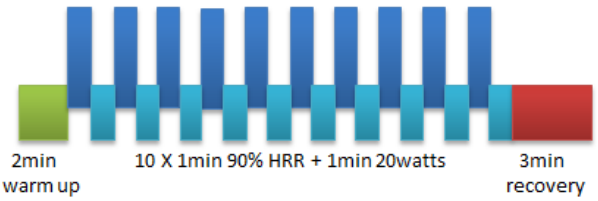
HIIE visit (1 for males, 2 for females): This is the high intensity interval exercise session (HIIE) and the protocol will follow this pattern: 1 minute at 90% of your maximal heart rate based on the maximal value obtained in your fitness test (VO_{2max} test), followed by 1 minute easy cycling in a low resistance (20 watts), for 10 times. The actual exercise protocol will be last 20 minutes, while 2min warm up and 3min recovery in a low resistance (20 watts) will be included (total duration of exercise 25 minutes). Artery function of your arm and blood samples will be taken pre, 10 min, 1h and 2h post exercise. This visit will be last approximately 3 hours.

24h post-exercise visit (2 for males, 4 for females): This visit will be 24 hours after the exercise visits. Blood sample and artery function will be assessed. This visit will last approximately 30 minutes.

Figure explanation for exercise protocols MICE and HIIE:



MICE protocol



HIIT protocol

Further explanations for the tests:

Artery function: This test is called flow-mediated dilation (FMD) and is a non-invasive method to measure the function of the artery in your upper arm (brachial artery) by using an ultrasound scan. This is the same technology used to scan pregnant women. You will rest for 15 minutes in the supine position and a blood pressure cuff will be placed around your lower arm. The cuff will then be inflated to a high pressure for 5 minutes, and then the cuff will be released and your artery will be continuing scan for further 3 minutes.

Blood samples: A cannula (small flexible tube) will be placed into a forearm vein of one arm to allow us to obtain blood samples at various time-points. Specifically, a small blood sample (15ml) will be taken 4 times (before, 10min, 1h, and 2h post exercise) during exercise visits (2 exercise visits for males/4 exercise visits for females), and 1 time in 24h post exercise visits (2 exercise visits for males/4 exercise visits for females). We will analyse certain biomarkers contained within your blood including those which will tell us about the oxidative stress, antioxidant capacity and platelet function after an acute bout exercise. Analysis should be conducted to LJMU. Blood samples will be retained for potential future analysis of additional biomarkers.

4. Are there any risks/benefits involved?

This study does **NOT involve major risks.** The most obvious risks to you involve blood sampling, exercise and cuff inflation during vascular assessment. Inflation of the blood pressure cuff during the FMD may cause a slight sensation of "pins and needles" in your arm, but this will disappear when the cuff is deflated, and it is not dangerous in any way. Blood samples will be taken on several time-points during some visits. However no additional risks are related to the number of blood samples, as cannula will be placed into the vein and kept there during the visit. All blood samples will be taken from this cannula. You will feel a sharp pain when the needle is inserted but this will be short-lived. The researchers are also experienced in this technique so the pain experienced will be minimal. You may also develop a small bruise on your arm, which can be prevented by applying pressure on the arm when the cannula has been taken out (the researcher will remind/instruct you to do this). The exercise protocol, especially HIIE session can make you feel tired, but it is the same as regular feeling after training and is not enough to cause you pain. At **any time** during the experiment, you are free to voluntary terminate the procedure and stop the experiment.

There are no potential direct benefits from participating in this study, although you will have **an assessment of your exercise performance and your cardiovascular system.** If you wish, we **can give you some oral advice regarding your exercise training, based on your personal data.** In addition, you will **have the opportunity to be exposed to current and cutting edge research tools** that will allow you to understand the effects of exercise on the cardiovascular system while you exercise. Finally, the information that we gain thought this study will help us to know more about the impact of a single bout of exercise on cardiovascular function.

5. Will my taking part in the study be kept confidential?

We would like to emphasise that your confidentiality is being safeguarded by the researchers prior to, during and after the study. The laptops, PCs and external drives on which data is stored are password protected. Data will be kept anonymous using unique identification codes. Hard copies of any personal data will be stored in a locked filing cabinet.

It is important to note that taking part is voluntary and you can withdraw from the research at any stage. There will be no negative consequence if you choose to withdraw.

This study has received ethical approval from LJMU's Research Ethics Committee. *(REC reference number and date of approval will be inserted when this study will be approved)*

If you are interested to participate in this study and/or would like to ask more information regarding the project, please contact me via email or phone.

Contact Details:

Miss Andrea Tryfonos
Research Institute for Sport & Exercise Sciences
Liverpool John Moores University
Byrom Street
Liverpool
L3 3AF

A.Tryfonos@2016.ljmu.ac.uk
0151 904 6278

Dr Ellen Dawson
Research Institute for Sport & Exercise Sciences
Liverpool John Moores University
Byrom Street
Liverpool
L3 3AF

E.Dawson@ljmu.ac.uk
01519046264

If you any concerns regarding your involvement in this research, please discuss these with the researcher in the first instance. If you wish to make a complaint, please contact researchethics@ljmu.ac.uk and your communication will be re-directed to an independent person as appropriate.