THE YLEANA ARCE FOUNDATION

MIN | EX
SEX DIFFERENCES IN MINIMUM DOSE OF EXERCISE RESPONSE

Northumbria University NEWCASTLE

BRIEF OVERVIEW

Sex differences in the minimum effective dosage of exercise for exerkine release

WHO CAN TAKE PART

- Adults aged 18–45
- Train aerobically 2+ times a week
- Generally healthy, no heart or metabolic conditions
- Females: regular menstrual cycle, no hormonal contraception

YOUR VISITS

S — Screening visit
Eligibility check, baseline measurements, a fitness test, and you're set up with an activity tracker and food diary to complete at home.

4 — Four exercise visits
One interval session and three treadmill runs to exhaustion at different speeds, in a random order. Samples are collected before and after each.

F — Females only: one extra visit
A repeat of the interval session during a different phase of your menstrual cycle, timed using home ovulation tests.

5 visits total for males, 6 for females. Each visit is spaced at least 2 days apart.

TIME COMMITMENT

Screening visit	Each lab visit	Same time every morning
~1h 30m	~4h total	All lab visits start at 08:00

WHAT HAPPENS AT EACH EXERCISE VISIT

- **Blood sample**: taken 9 times across the visit, via a small tube in your arm
- **Saliva**: a drool spit sample, 4 times
- **Urine**: 3 times
- **Skin fluid**: a painless suction patch on your forearm, up to 6 times

COMFORT AND SAFETY

- Safety harness worn on the treadmill at all times
- Trained researchers supervise every visit
- You can stop or withdraw at any time, no reason needed
- Your data and samples are anonymised and kept confidential

Thank-you payment
£18.50 per visit, paid by BACS transfer

£92.50–£111
for completing the study

Study Title

Sex differences in the minimum effective dosage of exercise for exerkine release

Investigator: Dr Paul Ansdell

Participant Information Sheet

You are being invited to take part in this research study. Before you decide it is important for you to read this leaflet so you understand why the study is being carried out and what it will involve.

Reading this leaflet, discussing it with others or asking any questions you might have will help you decide whether or not you would like to take part.

What is the Purpose of the Study

When you exercise, your body releases chemical messengers into your bloodstream called "exerkines." These molecules are thought to be responsible for many of the health benefits of exercise, such as reduced inflammation, improved brain health, and better blood sugar control. However, we still don't know very much about how different types of exercise affect their release.

This study aims to find out which types of high-intensity exercise produce the strongest exerkine response. You'll complete one screening day and four (five if you are female) different running sessions on a treadmill: one involving repeated intervals and three involving continuous running at different speeds until you can't continue. We'll collect blood and other body fluid samples before, during, and up to three hours after each session to track how exerkine levels change over time.

We also want to understand whether men and women respond differently, and whether a woman's menstrual cycle phase influences the exerkine response. Additionally, we're exploring whether exerkines can be detected in saliva, urine, and fluid just beneath the skin, which could one day replace the need for blood tests. Finally, we'll use advanced molecular profiling techniques to search for previously unknown exerkines that may play important roles in how exercise improves health.

Why have I been invited?

You've been invited because you are a healthy adult aged between 18 and 45 years who exercises regularly at a recreational level. This study needs participants who are physically active enough to safely complete high-intensity treadmill running sessions but are not elite or competitive athletes. We are recruiting equal numbers of males and females, 22 of each, to properly compare how biological sex influences the body's response to exercise.

If you are female, we are also investigating how the menstrual cycle affects the release of exercise-related molecules. For this reason, female participants will complete one additional exercise session during a different phase of their cycle, allowing us to compare responses when hormone levels are naturally low versus when they are higher.

Before taking part, you'll attend a screening visit where we'll check that you meet all the eligibility criteria, including measures of your general health, fitness, and, for females, your menstrual cycle regularity. This helps us ensure the study is safe for you and that the results are scientifically reliable.

Do I have to take part?

Your participation is entirely voluntary. It is up to you whether you take part in the study. This information sheet is given to you to help you make that decision. If you decide to take part, you can stop testing and discontinue participation at any point without any need for explanation. You are completely free to decide whether or not to take part, or to take part and then leave the study before completion. If you decide to take part and later withdraw you will still receive **£18.5** for each completed visit

What will happen if I take part?

If you agree to take part, you will attend our laboratory on six occasions (five for males). Prior to your first visit if you are female you will be asked to use home ovulation test kits for one menstrual cycle. At your first visit, we'll check your eligibility, take some baseline health measurements, and ask you to complete a maximal treadmill running fitness test. The maximal fitness test will be used to set the speeds for the following sessions so they are individualised to you. You'll also be given a wrist-worn activity monitor to wear for seven days, a food diary to complete online, and an optional kit for collecting stool samples at home.

You will then return for four separate exercise sessions, each involving high-intensity treadmill running in a different format. The order of these sessions will be randomly assigned. At each visit, we'll collect small samples of blood, saliva, urine, and fluid from just beneath the skin at several time points; before exercise and at intervals up to three hours afterwards. Each visit will last approximately four to five hours in total, and visits will be spaced at least two days apart. Based on measurements during the screening test, we will ask you to target an estimated caloric intake.

If you are female, you will attend one additional visit to repeat one of the exercise sessions during a different phase of your menstrual cycle. You will also be asked to use home ovulation test kits to help us schedule this visit at the right time. All visits will take place in the morning after an overnight fast.

Detailed procedures

At each exercise visit, you will arrive at the laboratory in the morning having fasted overnight, avoided exercise for

at least 48 hours, alcohol for 24 hours, and caffeine for 12 hours. You'll be asked to track your macronutrient consumption and food intake the day prior to the first lab visit and requested to repeat the same food intake before each subsequent lab visit. Please bring runners, light weight sportswear (which doesn't cover or can be pulled to reveal the elbow and lower arm), and a hoodie or sweater which can be worn over your sportswear.

On arrival, we'll measure your blood pressure, heart rate, and blood, lactate, glucose and haemoglobin using a small earlobe-prick sample. A small plastic tube called a cannula will then be placed into a vein in your arm so that blood samples can be taken at multiple time points without repeated needle insertions. Approximately 10 millilitres of blood will be drawn at each time point. We'll also collect saliva by asking you to gently spit into a tube, urine samples into a sterile container, and a small amount of fluid from just beneath the skin on your forearm using a painless microneedle device that applies gentle suction for around 15 minutes.

You'll then complete one of four exercise sessions. Three involve continuous running at a set speed until you can no longer continue, lasting roughly 2, 6, or 15 minutes, depending on the speed. The fourth involves four rounds of 4-minute hard running with 3-minute recovery jogs in between. Throughout all sessions, your breathing, heart rate, and effort level will be monitored, and you'll wear a safety harness attached to the treadmill.

After exercise, you'll remain in the laboratory while we continue collecting samples at regular intervals: immediately after stopping, then at 15, 30, 45, 60, 90, 120, and 180 minutes. You are free to drink water throughout, and the research team will record how much you consume.

What are the possible disadvantages of taking part?

Aside from taking the time out of your life to participate, which we are extremely grateful for, there are relatively few disadvantages.

The exercise sessions are deliberately high-intensity, and in three of the four sessions you will be asked to run until you physically cannot continue. This will be strenuous and uncomfortable, and you may feel muscle soreness, fatigue, or light-headedness afterwards. However, all sessions will be supervised by trained researchers and you will wear a safety harness attached to the treadmill at all times to prevent any risk of falling. Should you experience too much discomfort and wish to stop a session early or withdraw from the study entirely, that is perfectly acceptable.

A cannula will be placed into a vein in your arm at each exercise visit to allow us to take repeated blood samples without multiple needle insertions. This may cause temporary discomfort, bruising, or minor swelling, and there is a small risk of infection. The researchers inserting the cannula are fully trained, and we will be using standard clinical methods with strict sterile procedures throughout.

Earlobe-prick blood samples may cause brief, mild pain. The microneedle device used to collect fluid from beneath the skin on your forearm uses gentle suction and may cause mild, temporary discomfort or slight redness, but this is short-lived.

The study also requires a significant time commitment. You will need to attend the laboratory on five or six occasions, each lasting several hours. Before every visit you must fast overnight, avoid exercise for 48 hours, alcohol for 24 hours, and caffeine for 12 hours, which may disrupt your normal routine. If you are a woman, you will also need to use home ovulation test kits and track your menstrual cycle across multiple months, which adds a further practical demand. We appreciate that this is a lot to ask and will do our best to schedule visits at times that work for you.

What are the possible benefits of taking part?

You will receive your individual results from the fitness test ($\dot{V}O_2$peak and running speed), along with other physiological measures such as lean body mass, fasting blood glucose, and blood pressure. If you are female, you can also request your hormonal profile data, along with any other data collected about you, upon request following the study.More broadly, your participation will help address the current lack of research into female exercise physiology, and will contribute to the development of non-invasive biomarker sampling methods (saliva, urine, interstitial fluid) with potential applications in both high-performance sport and general health.In addition, to thank you for your participation and for kindly giving up your time upon completion of each visit you will be given **£18.50** via BACS transfer for a total of **£92.50** for males and **£111** for females.

How can I withdraw from the study?

If you do wish to withdraw then you can do so without any judgement or negative consequences. Simply email any of the research team informing us that you do not wish to continue with testing. You do not have to give any reason.

Will my taking part in this study be kept confidential and anonymous?

All data will be dealt with under the strictest of guidelines and according to the Data Protection Acts of 1984 and 1998, and General Data Protection Regulation (GDPR) 2016. All data will remain anonymous other than to the researcher and supervisor. All data collected will be kept on a secure password protected computer system. Your name will not be written on any of the data we collect; the written information you provide will have an ID number, not your name. The consent form you have signed will be stored separately from your other data, in a locked filing cabinet. The data collected from you in this study will be confidential.

How will my data be stored?

All data will be stored in accordance with University guidelines, the Data Protection Act and General Data Protection Regulation. Data will be stored on a password protected computer and backed up onto a password protected cloud storage service that will only be accessible to the research team. Information will be stored for 3 years from completion of the study.

What will happen to the results of the study?

The results of the study will be used to formulate relevant conclusions. The general findings might be reported in a scientific journal or presented at a research conference, however the data will be anonymised and you or the data you have provided will not be personally identifiable.

Who is Organising and Funding the Study?

This study is organised and funded by Northumbria University and the Yleana Arce Foundation.

Who has reviewed this study?

This study and its protocol have received full ethical approval from the Chair of the Faculty of Health and Life Sciences Ethics Committee. **Project ID: 12241**. If you require confirmation of this, please contact the Chair of the Faculty of Health and Life Sciences Ethics Committee (Dr Claire Thornton claire.thornton@northumbria.ac.uk) stating the full title and principal investigator of the study.

Contact for further information:

You can find out more about how we use your information at: www.northumbria.ac.uk/about-us/leadershipgovernance/vice-chancellors-office/legal-services-team/gdpr/gdpr---privacy-notices/ or by contacting a member of the research team.

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