Investigating the possible link between habitual diet, physical activity, sleeping patterns, obesity status and age with gut bacterial composition, gut barrier function, metabolic endotoxemia, systemic inflammation and glycemic control.

Adult Participant Information Sheet

Investigators Details:

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Dear potential participant,

Thank you for your time! We would like to invite you to take part in our study. Before you make your decision, it is important for us that you understand why the research is being done and what it would involve for you. Someone from our team will go through the information sheet with you and answer any questions you have. Talk to others about the study before deciding if you wish.

What is the purpose of the study?

In the UK, 25% of the adults are affected by metabolic syndrome (NHS, 2016). Metabolic syndrome is a cluster of different conditions including: hyperglycaemia, insulin resistance hypertriglyceridemia, dyslipidaemia and hypertension. Such individuals also have increased risk of developing type 2 diabetes and cardiovascular disease. The factors contributing to the development of metabolic syndrome are potentially numerous and understudied in humans, with much of what we think we know coming from animal research.

Recent animal studies have pointed towards gut health playing a role in metabolic health. More specifically it has been suggested that changes in the composition of the gut microbiota may drive insulin resistance and type 2 diabetes through a mechanism that is linked to increased gut permeability and the development of metabolic endotoxemia and inflammation. Yet, this link has not been confirmed in humans, so we are proposing a study to determine a possible relationship. Given that the composition of the gut microbiota can be influenced by diet, physical activity, age, stress, sleep pattern etc. we propose to study all these potential influencers.

Who is doing this research and why?

This project is being led by Dr Carl Hulston (Senior Lecturer, Loughborough University), Dr Oonagh Markey (VC Research Fellow, Loughborough University), Dr Nicolette Bishop (Reader, Loughborough University), Miss Malvina Begalli (PhD researcher, Loughborough University), and Miss Amber Leonard (PhD researcher, Loughborough University). This research will look at the relationship between diet, physical activity, sleeping patterns, obesity status and age etc. and measures of gut bacterial composition, gut barrier function and metabolic health.
This study will provide us with new insights on the effect of different physiological and behavioral/ lifestyle variables on gut health and metabolic function.

Can I take part? (Are there any exclusion criteria?)

The current inclusion criteria include:

- Men and women
- 18-70 years old
- Body mass index (BMI) = 18.5- 40.0 kg/m^2
- Non-smokers (including the use of vaporisers and e-cigarettes),
- Currently not taking anti-inflammatory drugs (excluding aspirin)
- No cardiometabolic (e.g. heart disease, high blood pressure) or inflammatory illness
- Not taking antibiotics and antimicrobial drugs for at least three months
- Both physically active and sedentary individuals will be eligible to take part in the study.

What will I be asked to do?

The total time commitment of the study is approximately 10 hours. You will be asked to attend the research laboratories within the Clyde Williams Building at Loughborough University on the three occasions:

1. Pre-screening visit (1h)

You will be asked to provide us with the signed informed consent. During the visit, basic measurements of height, body mass, hip and waist circumference will be taken and you will be asked to complete a questionnaire concerning your health.
Before leaving you will be provided with a pedometer and a sleep diary: you will wear the pedometer and complete the sleep diary during the 7 days prior the following visit. You will be provided also with faecal and urine collection kits, which need to be used in close proximity to the following visit, ideally the evening before or morning of the visit, depending on toiletry habits.

2. *First experimental visit* (3h)

You will be asked not to consume any alcohol or caffeine (coffee, tea, supplements containing caffeine). In addition, we ask you to abstain from vigorous exercise during the 24 hours before the visit. We will ask you to come to the laboratory in the morning after a 12 hour overnight fast. After voiding, being weighed and providing us with the home-collected faecal and urine samples, you will rest for 20 mins and then your blood pressure will be measured. Next, you will be asked to lie down in a temperature-controlled room. While lying down on a couch bed, a cannula (which is a thin flexible tube) will be inserted into a vein in your arm, and this will remain in place with minimal discomfort to allow us to take blood samples during the study day. A 55 ml blood sample (volume equivalent to three tablespoons) will then be taken. Then you will ingest a

standard oral glucose challenge solution (75 g of glucose in 300 ml water) and further blood samples of 10 ml each will be taken at 15, 30, 45, 60, 90, and 120 min after ingestion. During this 2-hour period, you will complete questionnaires concerning your food habits (food frequency questionnaire), as well as your habitual physical activity and mood-state. After this the cannula will be removed and your body composition will be assessed through bioelectrical impedance analysis [BIA; body composition analysis]. Lastly, you will be asked to perform a timed sit-to-stand movement test to assess your health.

3. *Second experimental visit* (5.5h)

On a separate day you will return to the laboratory for the second and final experiment. Again, you will be asked not to consume any alcohol or caffeine (coffee, tea, supplements containing caffeine). In addition, we ask you to abstain from vigorous exercise during the 24 hours before the visit. We will ask you to come to the laboratory in the morning after a 12 hour overnight fast. After voiding and being weighed, you will ingest 10 g of two simple sugars (5 g of lactulose and 5 g of mannitol) dissolved in 200 ml of plain water. For the next 5 hours, you will be asked to collect all urine output in containers we provide. A further drink (300 ml of plain water) will be provided at 3 h to stimulate additional urine output. During this timeframe, you will complete any of the questionnaires remaining from the previous visit. After 5 h you will be free to leave the laboratory.

Once I take part, can I change my mind?

Yes! After you have read this information and asked any questions you may have if you are happy to participate, we will ask you to complete an Informed Consent Form, however if at any time, before, during or after the sessions you wish to withdraw from the study please contact the main investigator. You can withdraw at any time, for any reason and you will not be asked to explain your reasons for withdrawing.
After you have completed the study, you will still be able to withdraw your data, but only before these data have been aggregated or published, which is likely to be within 3 - 6 months.

Will I be asked to attend any sessions and where will these be?

Yes, you will be required to attend the 3 sessions described above, days and time will be organized according to your availability. If you have any further queries, questions or concerns at any point, one of the investigators will be happy to meet with you and explain everything you are curious to know. All the visits will be held in Clyde Williams Building of the School of Sport, Exercise and Health Sciences.

How long will it take?
The total time commitment we asked from you is approximately 10 hours, distributed in three visits of respectively 1, 3 and 5.5 hours. Days and time can be arranged according to your availability.
Are there any disadvantages or risks in participating?

All of the procedures involved in this study are low risk and our screening procedure is designed to ensure that you will only participate if it is safe for you to do so. A first responder will be present at all study visits and will seek medical help immediately, in the event of an adverse event. Blood samples will be taken by qualified phlebotomists (blood takers). The procedure for taking blood directly from a vein in your arm is

routine. Blood sampling via a flexible cannula may cause minor bruising, an inflammation of the vein or hematoma (a small accumulation of blood under the skin). Good practice, however, minimises this risk and there is much less discomfort from a single cannulation than repeated venipunctures throughout the day. Some people may feel faint when they give blood. The volume of blood collected during each study visit is approximately 115 ml (less than 1/4 pint over the whole day), will cause no adverse consequences.

Data Protection Privacy Notice

Loughborough University will be using information/data from you to undertake this study and will act as the data controller for this study. This means that the University is responsible for looking after your information and using it properly. Loughborough University will keep identifiable information about you for 10 years after the study has finished. The University's Data Protection Officer can be contacted at: dp@lboro.ac.uk.

What personal information will be collected from me?

We will be collecting personal information including your name, contact details and gender. You will be also asked to complete questionnaires concerning your health, physical activity, mood, sleep pattern and nutritional habits. Last, we will collect sensitive information on your health (through a health questionnaire) and ethnicity (through ethnicity questionnaire).

Will my taking part in this study be kept confidential? How long will my personal data be retained?

Yes – These data will be collected and stored in strict accordance with the General Data Protection Regulations. All personal information will be kept confidential and the results will be anonymised.
On entry to the study you will be assigned a study ID number and all of your data will be associated with this number and not by name. All study data will be kept securely and only accessible to the responsible investigators. All study data and associated samples will be disposed of within 10 years.

How will the data collected from me be used?

Whilst completing the consent form, you will be asked if you agree for bodily samples taken during this study (i.e. blood, urine and faecal samples) to be stored for future research in the same research these as the project. However, you can opt in/out of this when completing the consent form. All study data and associated samples will be disposed of within 10 years.

What is the legal basis for processing the data?

Personal data will be processed on the "public task basis" this means that we as researchers at Loughborough University process personal data for the public good, and the existing systems by which you consent to participate in this study give you control over how your data is used.

Under the General Data Protection Regulation (GDPR), some of the personal data which will be collected from you is categorised as "sensitive data". The processing of this data is necessary for scientific research in accordance with safeguards. This means that study has gone through an ethical committee to ensure that the appropriate safeguards are put in place with respect to the use of your personal data.

Will my data be shared with others?

Only the researchers involved in the study will be able to access the data.

How long will the anonymised data/samples be retained?

All data will be dealt with under the strictest of guidelines and according to the General Data Protection Regulations. All data will be kept for 10 years and then destroyed. Electronic data will be stored on the investigators encrypted hard drive with a passcode required, and hard paper data will be stored in a locked office.

What will happen if you find abnormal results when testing my blood samples?
With your permission and following a discussion with you, we would send a copy of the blood test results to your GP so that they can provide any further advice that they deem necessary.

I have some more questions; who should I contact?
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What if I am not happy with how the research was conducted?

If you are not happy with how the research was conducted, please contact the Secretary of the Ethics Approvals (Human Participants) Sub-Committee, Research Office, Hazlerigg Building, Loughborough University, Epinal Way, Loughborough, LE11 3TU. Tel: 01509 222423. Email: researchpolicy@lboro.ac.uk

The University also has policies relating to Research Misconduct and Whistle Blowing which are available online at http://www.lboro.ac.uk/committees/ethics-approvals-human-participants/additionalinformation/codesofpractice/ .

If you have taken steps to have a concern or complaint about Loughborough University's handling of data resolved but are still not satisfied you have a right to lodge a complaint with the Information Commissioner's

Office (ico), who are the relevant regulator for data privacy and protection matters. The ico can be contacted at Wycliffe House, Water Lane, Wilmslow, SK9 5AF and your will find more information at https://ico.org.uk