FMHS RESEARCH ETHICS COMMITTEE (FMHSREC)
Ethics Application Form/Protocol
Intervention/ Basic Science/Physiology Studies in Healthy Volunteers

ONLY FULLY SIGNED (electronic) TYPE-WRITTEN APPLICATIONS WILL BE ACCEPTED, BY EMAIL

Please complete this form if your study involves the administration of a licensed drug, herbal remedy, food supplement or physiological intervention/ testing in healthy volunteers AND is NOT a clinical trial.

Should you require any assistance in completing this document, please contact the REC administrator in the first instance: FMHS-ResearchEthics@nottingham.ac.uk

STUDY DETAILS	
Full study title	Influence of the menstrual cycle on muscle and liver glycogen and circulating substrates during exercise in healthy women
Short title	IMCOM
FMHS REC reference	
Date and version number	05.08.2021 V1.0
Principal investigator	Professor Guruprasad P Aithal
University email contact	Guru.aithal@nottingham.ac.uk
Student (if applicable)	
University email contact	Stephen.bawden@nottingham.ac.uk
University telephone contact	+44 (0)115 7487053
Medically qualified collaborator (licensed doctor)	Professor Guruprasad P Aithal
Sponsor	The University of Nottingham
External funding	This study is funded by University of researcher Tomoka Matsuda and support from NIHR Nottingham BRC.
Will you submit or have you submitted this study to another ethics committee?	Yes ☐ No ☒
If other relevant approvals for this research are required (e.g. from other universities' ethics committees) please attach them and give more details below:	
N/A	
Declaration of any conflicts of interest	none
Confidentiality statement	This document contains confidential information that must not be disclosed to anyone other than the authorised individuals from the University of Nottingham, the Investigator Team and members of the Faculty of Medicine & Health Sciences University Research Ethics Committee (FMHS REC), unless authorised to do so.

RESEARCH TEAM	
Principal investigator (PI) title and name, job title	Professor Guru Aithal Professor of Hepatology
Department/institute name	Nottingham Digestive Diseases Centre Queens Medical Centre University of Nottingham
Role in study	Study PI. Previous experience of managing human *in vivo* research projects in healthy volunteers and patients.
Training/qualification in research ethics	GCP training, UoN Code of Research Conduct and Research Ethics
Co- investigator (Co-I) title and name, job title	Professor Penny Gowland Professor of Physics
Department/institute name	SPMIC, Department of Physics and Astronomy University of Nottingham
Role in study	Protocol advise, MRI expertise, Previous experience of managing human *in vivo* research projects in healthy volunteers and patients.
Training/qualification in research ethics	GCP (2021), Code of Research Conduct and Research Ethics
Co- investigator (Co-I) title and name, job title	Dr Jane Grove Assistant professor
Department/institute name	Faculty of Medicine & Health Sciences, University of nottingham
Role in study	Protocol support and advice
Training/qualification in research ethics	GCP, UoN Code of Research Conduct and Research Ethics
Co- investigator (Co-I) title and name, job title	Dr Stephen Bawden Senior Research Fellow
Department/institute name	SPMIC, Department of Physics and Astronomy University of Nottingham
Role in study	Study design, Scanner operation, MRI development, data acquisition and analysis, publication write up
Training/qualification in research ethics	GCP (2021), UoN Code of Research Conduct and Research Ethics
Co- investigator (Co-I) title and name, job title	Ms Tomoka Madsuda Research Student
Department/institute name	Graduate School of Health and Sport Science Nippon Sport Science University, Tokyo, Japan
Role in study	Researcher, Study design, Data acquisition, Analysis, Publication write up
Training/qualification in research ethics	
Co- investigator (Co-I) title and name, job title	Dr Mehri Kaviani Scanner operator
Department/institute name	SPMIC, Department of Physics and Astronomy University of Nottingham
Role in study	Researcher, Data acquisition, Analysis, Publication write up
Training/qualification in research ethics	GCP (2021), UoN Code of Research Conduct and Research Ethics

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1. SYNOPSIS

Please state why this study is not considered a clinical trial	A pilot study which involves healthy adult research volunteers (not NHS patients) and has non-clinical, descriptive end-points.	
List all sites where this study will be conducted	SPMIC, University Park	
Age range of study participants	18 – 35 years	
Planned sample size and groups	12 participants, each undertaking 5 visits	
Planned study duration	1 years	
Anticipated start date	08/2021	
Anticipated end date	08/2022	
	Objectives	**Outcome Measures**
Objectives and outcome measures	PRIMARY OBJECTIVE • Investigate variations in moderate exercise-induced change in hepatic glycogen throughout the menstrual cycle SECONDARY OBJECTIVES • Investigate variations in moderate exercise-induced change in muscle glycogen throughout the menstrual cycle • Investigate the relationship between liver glycogen, muscle glycogen, and plasma index (estradiol, progesterone, blood lactate, blood glucose, free fat acids) throughout the menstrual cycle.	PRIMARY ENDPOINT • Liver glycogen concentration evaluated using 13C MRS. SECONDARY ENDPOINTS • Muscle glycogen evaluated using 13C MRS • Blood biomarkers: Metabolites (glucose, lactate and lipids), hormones (Estradiol, Progesterone, blood lactate, blood glucose, free fatty acids.)
Name of drug/ substance/ intervention	Moderate exercise (60 minutes at 70% max heart rate)	
Purpose of drug/ substance/ intervention use in this study	Reduce hepatic and muscle glycogen stores	
Adverse reactions and side effects posing a particular risk with this treatment	None in healthy participants	

2. ABBREVIATIONS

CRF	Case Report Form
GCP	Good Clinical Practice
PI	Principal Investigator at a local centre
R&D	Research and Development department
MRS	Magnetic Resonance Spectroscopy
SPMIC	Sir Peter Mansfield Imaging Centre
E-FP	Early Follicular Phase
L-FP	Late Follicular Phase
LP	Luteal Phase
W_{max}	Maximum workload
CRF	Case Report Form
GCP	Good Clinical Practice
PI	Principal Investigator at a local centre
R&D	Research and Development department
MRS	Magnetic Resonance Spectroscopy

3. BACKGROUND AND RATIONALE

Background

In recent years, the activities of female athletes have been increasing. However, the blood concentrations of female hormones change significantly for premenopausal women throughout the menstrual cycle (1). Therefore, since the condition of the body changes with the menstrual cycle, it is necessary to establish a conditioning method that considers the menstrual cycle.

Previous work undertaken by co-investigator at Nippon Sports University (Tokyo) has shown that oxidative stress and serum carnitine, which are physiological indices highly related to fatigue, differ through the menstrual cycle (2,3). In addition, studies suggest that the menstrual cycle influences muscle glycogen utilization during high-intensity intermittent exercise until exhaustion in women with habitual exercise activity (European Journal of Sport Science, under revision). Estrogen promotes increased fat oxidation, sparing muscle and hepatic glycogen, decreasing gluconeogenesis, and increasing the exercise time until exhaustion (4). Progesterone is known to reverse several estrogenic effects, including reducing fat oxidation (5). The amount of glycogen stored in the body, either in liver or muscle, is highly related to fatigue and exercise performance. However, the study of a 30-kg box lifting and transport exercise is the only study of the menstrual cycle and liver glycogen by human (6). Furthermore, the study has compared in the early follicular phases (E-FP) when both of serum estradiol and progesterone concentrations are low with in the luteal phase (LP) when both of serum estrogen and progesterone concentrations are high; however, the LP is characterized by high estradiol and progesterone concentrations, and it is thus not possible to determine the effects of estradiol alone. Therefore, conducting measurements in the late follicular phase (L-FP), when serum estradiol concentrations are high but serum progesterone concentrations are low, is necessary to confirm the effects of estrogen alone.

Aim: To investigate the effects of hormonal changes throughout the menstrual cycle on moderate exercise induced liver and muscle glycogen changes in healthy women.

4. PARTICIPANTS

Description of study participants
12 healthy females aged 18-35 with regular menstrual cycle (every 25 – 38 days), who take regular exercise. Participants must have no use of any oral contraceptives or other hormone medications for at least 6 months prior to the experiment.
Inclusion criteria
• Female • Age 18 – 35 yrs • Regular menstrual cycle (every 25 - 38 days). • Nulliparous • Habitually exercising (45 min moderate exercise, brisk walking or jogging, minimum 3 days per week) • Any ethnicity
Exclusion criteria
• No history of smoking, chronic diseases. • No use of any oral contraceptives or other hormone medications for at least 6 months prior to the experiment. • Pregnancy or lactation • Learning difficulties cognitive impairment, social problems, substance abuse or mental illness which would make it difficult to complete the protocol.
Recruitment
Participants will be recruited by posters placed in University of Nottingham buildings and staff areas of Queens Medical Centre; an electronic version of the study poster posted on University and local community social

media sites; and information included on the 'Call for participant' web site. The posters will include contact details of a member of the research team, for potential participants to make enquiries.

Those expressing an interest in the study will be sent a copy of the information sheet to read and, if they are then willing to participate, inclusion and exclusion criteria will be checked before an initial visit is arranged. They will be sent details of the initial appointment, along with directions, public transport information, and parking information, by email (or post if required).

It will be explained to all potential participants that entry into the trial is entirely voluntary and that they can withdraw at any time. In the event that they withdraw from the study it will be explained that their data collected up to the point of withdrawal cannot be erased and we will seek consent to use the data in the final analyses where appropriate.

Screening and eligibility assessment

All volunteers will be assessed to ensure that they are suitable to participate, following the criteria listed in the Inclusion / Exclusion Criteria sections. This will be checked with participants before the initial visit. To limit the demands on potential volunteers, participants will be offered to either come to a separate screening visit prior to the start of the study, or to fill our a screening questions over email with final checks undertaken on the initial visit.

Participants will be asked to complete the general study screening questionnaire and the MR Safety Screening questionnaire. Any individuals with tattoos will also be required to complete a tattoo safety form.

Information provided to participants and informed consent

An information sheet will be given to the participants, and informed consent will be obtained before any measurements are made and confirmed on the initial visit.

Participant confidentiality

Each participant will be assigned a unique trial identity code number, which will be used on Case Report Forms (CRFs), other trial documents, MR scans and the electronic database. CRFs will be treated as confidential documents and held securely in a locked cabinet. The investigator will make a separate confidential record of the participant's name, date of birth, and Participant Trial Number (the Trial Recruitment Log), to permit identification of all participants enrolled in the trial in accordance with regulatory requirements and for follow-up as required. This Trial Recruitment Log will be stored securely in a locked cabinet separately from anonymised records. Access to CRFs, database and other records shall be restricted to those personnel approved by the Principal or Lead Investigator, including relevant regulatory authorities, and recorded on the 'Trial Delegation Log'.

All research staff and investigators will endeavour to protect the rights of the trial's participants to privacy and informed consent, and will adhere to the Data Protection Act, 2018. The CRF will only collect the minimum required information for the purposes of the project. Computer held data, including the trial database, will be held securely and password protected. Access will be restricted by user identifiers and passwords (encrypted using a one-way encryption method).

5. STUDY PROCEDURES

Study Visits

The participants will be required to visit the research centre on 5 separate occasions, first for a preliminary set-up visit and subsequently at 4 points during the menstrual cycle. Each participant will be assigned a trial identity code number for use on trial documents, samples and the electronic database enabling anonymous

linkage of the bio-samples and data collected. At visits 2-5, a blood sample of up to 20ml will be collected by a researcher trained in venepuncture. During exercise visits, participants will be supervised by a researcher trained in basic life support and a qualified first aider will be present in the SPMIC in case participant feels faint or needs to immediately stop for any reason.

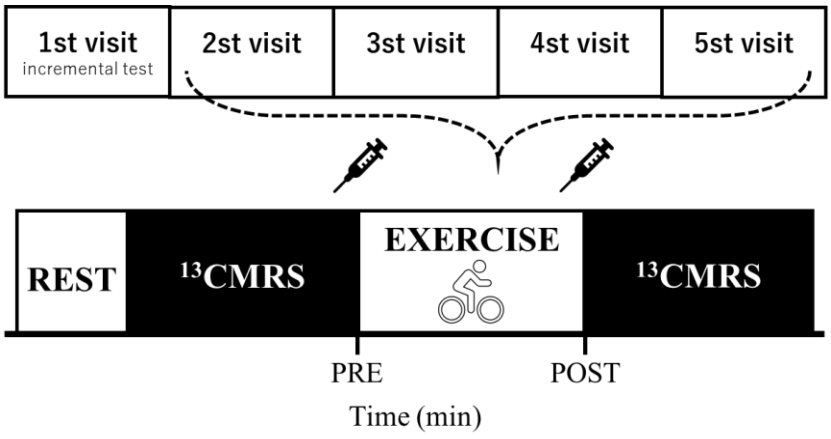
Participants will instructed to refrain from strenuous exercise on the day before visits, to maintain their normal diets, and to keep a diet diary for 2 days prior to each trial. All Participants consume the same standardized dinner on the evening before each test day, which will consist of a frozen tomato pasta vegetarian ready meal provided by the researchers.

VISIT 1 (INITIAL VISIT)

During the 1st visit their maximum workload capacity (Wmax) will be determined using a cycle ergometer. Participants will complete an exercise task for up to 2 hours, undergoing incremental steps of 3-4 minutes at constant speed (60 rpm) starting at 50 Watts and increasing by 25 Watts for at least 6 workloads until the participant cannot continue (exhaustion). Between each workload step, participants will have 3 minutes of rest to prevent early fatigue. Heart rate will be recorded for the last 10 seconds of each workload, averaged and plotted to determine the Wmax.

VISITS 2 – 5 (STUDY VISITS)

On the morning of visits 2-5, participants will be instructed to eat a standardized breakfast (2 slices of bread and jam without butter/margarine) at a fixed time, and then arrive at the Sir Peter Mansfield Imaging Centre (SPMIC) to begin scanning 3 hours after consumption, with 30 minutes rest at the SPMIC before initial scan. Baseline measures (Pre-exercise) of liver and muscle glycogen will be taken using 13C MRS along with blood samples for analysis. Each participant will then exercise on the cycle ergometer for 40 - 45 minutes at 70% Wmax based on heartrate which will be measured throughout the exercise (pedal speed kept at 60 rpm, adjusted workload). Immediately after exercise cessation (Post-exercise), participants will undergo 13C MRS measurement of liver and muscle glycogen levels, and blood samples is collected (below). Participants who are unable to complete the exercise regime will be removed from the study and paid and inconvenience allowance proportionally, as outlined in their information sheet and consent form.



Sample handling

Samples will be stored in a linked anonymised format and labelled using a combination of study reference, unique study identifier and cross referenced with location code numbers to permit accurate linkage to study data and the consent form. The master database will be held by the study investigators in a password encrypted file.

Blood samples will be processed in the Nottingham Digestive Diseases Centre (NDDC) laboratories, University of Nottingham (located in the QMC) or SPMIC laboratories (on University Park Campus). Samples will be

transported from SPMIC following local standard operating procedures and stored securely in the NDDC laboratories at -80°C prior to analysis. The analysis of samples will take place either in the NDDC laboratories, University of Nottingham or else samples will be shipped to commercial providers for specialist analyses with a material transfer agreement arranged.

Once the study is completed and analysis has taken place, any residual samples will be stored within the Nottingham Digestive Diseases Centre Biomedical Research Unit Research Tissue Bank for future research (DI William Dunn- Licence Number 12265) if participants are agreeable and sign the optional clause on the consent form. Where participants do not agree to the future use of the samples, they will be destroyed in accordance with the Human Tissue Act, 2004.

Standard operating procedures (SOPs) will be followed for all laboratory assays to ensure the high quality and reliability of our generated data.

Discontinuation/ withdrawal of participants from study

Participants may be withdrawn from the trial either at their own request or at the discretion of the Investigator. The participants will be made aware that this will not affect their future care. Participants will be made aware (via the information sheet and consent form) that should they withdraw the data collected to date cannot be erased and may still be used in the final analysis.

Definition of end of study

The end of participant involvement in the study is at the end of the MR scanning visit.

Expenses and benefits

This study requires participants to attend 5 visits in total, 1 initial visit and 4 weekly study visits. Each visit will last a full morning, requiring blood sampling and moderate exercise for one hour. An appropriate inconvenience allowance for this commitment of £75 per visit will be offered, and a further £25 on completion of the study, equalling a total of £400.

6. INTERVENTION(S) & DRUG ADMINISTRATION

a) Drug/ Substance/ Intervention 1

Name of drug/ substance/ intervention to be used	Moderate exercise
Formulation and route of administration for study	Wmax determined using incremental workload increases
Dose and route of administration for study	60 minutes at 70% maximum heartrate
Duration of treatment for study	1 x 60 minute of moderate exercise on each of 4 weekly visits
Licence status of this drug/substance	N/A
Usual indication	N/A
Usual dose	N/A
Usual duration of treatment	N/A
Where will drug/ substance be sourced from?	
Where will drug/substance be stored?	
How will drug/substance be dispensed?	

How will the drug/ substance be prepared by the researchers for use in this study?	

7. SAFETY

a) Definitions

Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a substance has been administered, including occurrences which are not necessarily caused by or related to that substance.
Adverse Reaction (AR)	An untoward and unintended response in a participant to a substance, which is related to any dose administered to that participant. A causal relationship between the administered substance and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the study intervention qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect. Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the study treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product set out in its summary of product characteristics (SmPC).

b) Reporting Procedures for Serious Adverse Events or Reactions

A serious adverse event (SAE) occurring to a participant should be reported to UoN FMHS UREC where, in the opinion of the PI, the event was 'related' (resulted from administration of any of the research procedures) and 'unexpected' (the type of event is not listed on this form as an expected occurrence). Reports of related and unexpected SAE's should be submitted by the PI within 7 days of the event. For fatal and life-threatening SUSARs, this will be done no later than 3 calendar days after the Sponsor or PI is first aware of the reaction. Any additional relevant information will be reported within 7 calendar days of the initial report.

c) Safety of Participants

What level of baseline screening will take place for this study?
• Self-reported medical history, including medication use will be taken. • Self-reported regular moderate exercise. • Participation screening questionnaire • MRI screening questionnaire

Provide details about the safety monitoring of participants and the staff/researchers carrying this out
When they are in the magnet, the participant will be given a button to push if they wish to speak with the operator, or if they wish to stop the scans at any point, and will be told to push the button if they feel uncomfortable, anxious or experience any unusual feelings such as heat, nausea, tremor or muscle twitches. MR scanner operators have undergone training in how to recognise, monitor and address side effects of MRS. Participants will be observed when they stand up after being in the magnet for an extended period of time and they will be told to inform the researchers if they feel lightheaded at any point. In the event that an individual does feel faint, first aid measures will be implemented. When the symptoms resolve, gradual position changes from supine to upright will be carried out with participant feedback elicited at each stage to determine any return of symptoms. Any adverse events that occur will be documented and filed in the participant's CRF and the event reported to the PI for review. Any serious adverse event will be documented and reported as described in section 7b.
Give details on the medical cover required and who will provide this cover
Study procedures do not require specific observation/monitoring by a medically-qualified professional. However, the PI (who is medically qualified) will be available by phone if researchers need support or advice, with nurses present for blood collection to assist and support researchers if required.
Will the participants' GP be informed about their participation in the study?
The participants' GP will not be informed about their participation in the study. However, details of the volunteer's GP will be taken at screening and they will be informed if any incidental findings are discovered
What is your planned procedure if an incidental finding is suspected?
If an incidental finding is discovered at screening or during brain imaging, initially this will be reported to the PI and the clinical significance of the finding assessed. Researchers will then inform the participant's GP if clinically significant and follow the wishes of the participant (sought at screening and documented on the SPMIC 'Abnormal Scans' form) regarding whether they wish for researchers to explain any findings to them directly, or for their GP to do this.

d) Ethical Considerations

Most research carries the risk of some ethical challenge. If this is the case you need to demonstrate your awareness of the problem and your response to mitigate ethical objections. There is a free text box below to detail any study specific ethical issues, however the following areas are often a cause for concern:

Will you include any vulnerable participants ? (e.g. children (under 16 years old) or vulnerable adults with a mental incapacity or prisoners) **If yes,** please describe how they are defined as vulnerable and detail any approved procedures or guidance that you feel apply.	Yes ☐	No ✓
Will taking part in the research put participants under any particular burden and/ or risk? **If yes,** describe how this will be mitigated.	Yes ☒	No
The length of time that the participant is in the scanner will be up to 1hr, which may become uncomfortable. Care is taken when initially positioning the participant on the scanner bed that they are made as comfortable as possible and regular contact with the participant is maintained during the scanning period to check on their welfare. Scan sequences can be halted to allow repositioning and the participant will be removed from the scanner at any point that they need a break. Moderate exercise is not considered burdensome given health and exercise screening of participants.		
Will the research involve deliberate *<u>deception</u>* of participants? **If yes**, justify why deception is used, describe deception and debriefing process, and include debriefing documents in the application.	Yes ☐	No ✓

Will any procedures affect your own or any member of the research teams physical and/ or psychological safety as a researcher? **If yes,** describe how this will be mitigated.	Yes ☐	No ✓
Does your research raise issues relevant to the Counter-Terrorism and Security Act (the Prevent Duty), which seeks to prevent people from being drawn into terrorism? **If yes,** please say how you plan to address any related risks.	Yes ☐	No ✓

Please give details of any other study-specific ethical and/or safety considerations.

Blood sampling carries usual risks associated with phlebotomy and extraction.

In 2020 and early 2021 *in vivo* human research was severely impacted by the Covid pandemic. Whilst we cannot predict the future, mitigation measures to protect staff and participants from Sars-Cov-2 transmission are in place within the SPMIC, and widespread vaccination of the UK population should contribute to keeping research activity possible going forwards. We will follow all government requirements and recommendations and adhere to University policies and best practice. Participants will be screened for covid symptoms via questionnaire before each visit.

8. STATISTICS AND ANALYSIS

Do you have a statistical plan? **If no,** please justify.	Yes ☒	No ☐

Number of participants & group allocations

12 participants each undertaking the same study regime

Have you done a sample size calculation? If yes, please give details below. If no, please give details to indicate you have considered the implications the selected sample size will have on the study outcome.	Yes ☒	No ☐

There is no data available on menstrual cycle effects on liver glycogen, so a formal sample size calculation is not possible. However, Hackney (7) found a significant increase in exercise induced depletion of muscle glycogen in the follicular phase compared to the luteal phase using muscle biopsy ($P < 0.05$) in a sample size of n = 8 (FP ΔGLYC = 46.4 ± 8.4 % vs LP ΔGLYC = 21.2 ± 16.3 %). Additionally, Casey et al (8) found a similar drop in liver glycogen in male participants following exercise.

The hypothesis of the present pilot study is that a similar reduction in glycogen utilization during the LP will be observed in the liver as in glycogen. As such, a sample size of n = 12 is a safe estimate to allow for variability in liver compared to muscle glycogen stores, and provide pilot data for future studies.

Analysis of Outcome Measures

MAGNETIC RESONANCE IMAGES AND SPECTRA ANALYSIS

Participants will be scanned on a 3T Philips Achieva system using a 12cm Pulseteq surface coil. Natural Abundance 13C MRS will be performed with standard block pulses and data fitted using jMRUI and in-house fitting algorithms written in Matlab. Glycogen concentration will be determined using an external reference and

phantom replacement and values before and after exercise compared (1 - add). The liver glycogen scan takes ~15 minutes and the muscle glycogen scan takes ~10 minutes.

BLOOD SAMPLE ANALYSIS

Samples will be stored in a linked anonymised format and labelled using a combination of study reference, unique study identifier and cross referenced with location code numbers to permit accurate linkage to study data and the consent form. The master database will be held by the study investigators in a password encrypted file.

Blood samples will be processed in the Nottingham Digestive Diseases Centre (NDDC) laboratories, University of Nottingham (located in the QMC) or SPMIC laboratories (on University Park Campus). Samples will be transported from SPMIC following local standard operating procedures and stored securely in the NDDC laboratories at -80°C prior to analysis. The analysis of samples will take place either in the NDDC laboratories, University of Nottingham or else samples will be shipped to commercial providers for specialist analyses with a material transfer agreement arranged.

Once the study is completed and analysis has taken place, any residual samples will be stored within the Nottingham Digestive Diseases Centre Biomedical Research Unit Research Tissue Bank for future research (DI William Dunn- Licence Number 12265) if participants are agreeable and sign the optional clause on the consent form. Where participants do not agree to the future use of the samples, they will be destroyed in accordance with the Human Tissue Act, 2004.

Standard operating procedures (SOPs) will be followed for all laboratory assays to ensure the high quality and reliability of our generated data.

STATISTICS

Statistical analyses will be conducted by Tomoka Matsuda, with the supervision and support of Professor Guru Aithal and Stephen Bawden. Data input, cleaning and analysis will be conducted using SPSS. Initially, an ANOVA f-test will be applied to the data comparing pre and post exercise values for each week of the menstrual cycle and any significant mains effect or interaction between exercise and week will be followed up by a means difference t-test. Change in glycogen during each week will also be analysed using a means difference t-test with significant changes ($p \leq 0.05$) reported.

Baseline characteristics of participants, outcome measures at baseline (liver fat content, blood biomarkers) and each period time-point will be summarized using descriptive statistics. Data will be presented as mean± standard deviation and the significance of difference will be set at $p < 0.05$. Any changes in the planned statistical methods will be documented in the trial report.

All data will be anonymised and analyses will be conducted on UoN computers/laptops or at Nippon Sports Science University. Uon data are regularly backed up to UoN servers and data will be stored for up to 7 years before being deleted.

9. DATA MANAGEMENT

It is now a University governance requirement that every research project has a **Data Management Plan (DMP)**: Here is the link to the required training course that will take 2-3 hours: DMP training

When you have completed the training a DMP is easy to create using DMPOnline at the following link: https://dmponline.dcc.ac.uk/ It is worth selecting the option for a research librarian if you have not completed the training. This should be appended to the end of this form.

Summary for Form

Who will have access to the *research* data?	Direct access will be granted to authorised representatives from the University of Nottingham and any host institution for monitoring and/or audit of the study to ensure compliance with regulations.
How will *research* data be stored?	Blinded data will be stored on a computer at the SPMIC and in Nippon sports university.
How long will *research* data be stored for?	Research databases and records will be retained for 7 years after the end of the study. Consent will be obtained from participants at screening to retain their fully anonymised (not linked) MR images for future research use.
What will be done with the *research* data at the end of the storage period?	At the end of the storage period, all paper records will be securely destroyed through the University 'confidential waste' stream. Electronically held records and data will be erased, but anonymised images may be retained, in the SPMIC archive beyond the 7-year time frame, for future research use if consent was obtained from participants to do so.
Who will have access to the participants' *personal* data?	Only members of the research team who need the information to conduct study activity will have access to the participants' personal data. Access will be prior approved by the PI and documented in the Delegation log.
How will *personal* data be stored?	Personal, identifiable data will be held securely in a locked cabinet separately to anonymised study data
How long will *personal* data be stored for?	To comply with the data protection act, personal data will be deleted as soon as possible after it is no longer needed for the study. Personal details e.g. those contained in the screening questionnaires and consent forms will be retained until the data have been analysed and interpreted. They will then be securely destroyed If the participant wishes to be informed of the results of the study, their contact details will be retained, and specific consent will be collected on the consent form.
Describe method(s) of research data entry/management	All study data will be entered on an Excel spreadsheet. The participants will be identified by a unique study specific number and/or code in any database. Their name or any other identifying detail will NOT be included in any study data electronic file. Any documentation with identifiable (personal) data will be stored securely and separate from the research data.

10. STUDY MONITORING AND OVERSIGHT

Who will be responsible for day-to-day supervision of the study?
Dr. Stephen Bawden
Give information about frequency of meetings that will be held to discuss progress/problems. Who will be present at the meetings?
A regular meeting will be held (~ 1 per month) with all or some of the PI and Co-Is present to share data and discuss progress, problems and dissemination.

11. ETHICAL AND REGULATORY CONSIDERATIONS

Declaration of Helsinki

The PI will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

Approvals

This form, an informed consent form, a participant information sheet(s) and any proposed advertising material will be submitted to the UoN FMHS REC for written approval. The research team will submit and, where necessary, obtain approval from the UoN FMHS REC and any other above-mentioned parties for all amendments to the original approved documents.

End-of-Study Report

The PI shall request, complete and submit an end-of-study report to the UoN FMHS REC within 6 months of the study end (participant involvement).

12. DISSEMINATION AND FEEDBACK OF STUDY OUTCOMES

Participant will be benefiting this area of research as the study will help us to advance understanding in this field and help to uncover more about the influence of menstrual cycle on glycogen. It will also provide a valuable resource for other researchers in future studies in the similar area of research. Results will be published in various scientific publications and academic peer reviewed journals. In addition, work will be presented at local and international academic conferences (e.g. European College of Sports science conference). Results will also be included as part of dissertation/thesis which will be publicly available. Participants will not be identified in any of these publications.

13. REFERENCES

Insert references used in text in number or alphabetical order (excluding those already listed in Section 4).

(1) Elliott-Sale KJ, Minahan CL, de Jonge XAKJ, Ackerman KE, Sipilä S, Constantini NW, et al. Methodological Considerations for Studies in Sport and Exercise Science with Women as Participants: A Working Guide for Standards of Practice for Research on Women. Sport Med. 2021 Mar 16;51(5):843–61.
(2) Matsuda T, Matsuda T, Furuhata T, Ogata H, Kamemoto K, Yamada M, et al. Effects of the Menstrual Cycle on Serum Carnitine and Endurance Performance of Women. Int J Sports Med. 2020;41(7):443–9.
(3) Matsuda T, Ogata H, Kanno M, Ishikawa A, Yamada M, Sakamaki-Sunaga M. Effects of the menstrual cycle on oxidative stress and antioxidant response to high-intensity intermittent exercise until exhaustion in healthy women. J Sports Med Phys Fitness. 2020;60(10):1335–41.
(4) Nicklas BJ, Hackney AC, Sharp RL. The menstrual cycle and exercise: Performance, muscle glycogen, and substrate responses. Int J Sports Med. 1989 Aug 14;10(4):264–9.
(5) Oosthuyse T, Bosch AN. The effect of the menstrual cycle on exercise metabolism: Implications for exercise performance in eumenorrhoeic women. Sport Med. 2010 Mar;40(3):207–27.
(6) Price TB, Sanders K. Muscle and liver glycogen utilization during prolonged lift and carry exercise: male and female responses. Physiol Rep. 2017 Feb 1;5(4):e13113.

(7) Hackney AC. Influence of oestrogen on muscle glycogen utilization during exercise. Acta Physiol Scand. 1999 Nov 167(3):273-4
(8) Casey A, Mann R, Banister K, Fox J, Morris P, Macdonald I, Greenhaff P. Effect of carbohydrate ingestion on glycogen resynthesis in human liver and skeletal muscle, measured by 13C MRS. Am J Physiol Endocrinol Metabl. 2000 278:E65-E75

14. DECLARATIONS AND SIGNATURES

I/ we, the researcher(s) agree:

- To start this research study only after obtaining approval from the UoN FMHS REC;
- To carry out this research study only if funding is adequate to enable it to be carried out according to good research practice and in an ethical manner;
- That it is the responsibility of the PI to ensure that all researchers working on this project are qualified and either experienced, or have received appropriate training (including ethical training), to conduct the research described;
- To provide additional information as requested by UoN FMHS REC before approval is secured and as research progresses;
- To maintain the confidentiality of all data collected from or about study participants;
- To notify UoN FMHS REC in writing immediately of any proposed change which would increase the risk or burden that any participant is exposed to, and await approval before proceeding with the proposed change;
- To notify UoN FMHS REC if the PI on the study changes and supply the name of the successor;
- To notify UoN FMHS REC in writing within seven days if any serious adverse event occurs in the course of research;
- To use data collected only for the study for which approval has been given;
- To grant access to data only to authorised persons; and
- To maintain security procedures for the protection of personal data, including (but not restricted to): removal of identifying information from data collection forms and computer files, storage of linkage codes in a locked cabinet, and password control for access to identified data on computer files.

Principal Investigator (Name)	Professor Guruprasad P Aithal
Principal Investigator (Signature)	
Medically qualified collaborator (Name)	Professor Guruprasad P Aithal
Medically qualified collaborator (Signature)	
Co-investigator (Name)	Dr Stephen Bawden
Co-investigator (Signature)	

CHECKLIST:

- Completed form signed by the PI
- Medically qualified collaborator indemnity where applicable
- Data Management Plan
- Participant information sheet (PIS)
- Informed consent form (ICF)
- Poster or advert or draft of text to be used in social media
- Letters of permission from gatekeepers, outside institutions where applicable
- Plan of action in case there is a problem or something of concern is raised
- Drug data sheets and/or information about food/substances being ingested/applied where applicable
- Standard protocols for procedures i.e. muscle biopsy, blood taking
- Safety information for any devices and procedures used where applicable
- Health/Safety Screening questionnaires
- For research being conducted overseas: an outline of the research ethics regulations, local laws and permissions, required
- Any additional relevant information

Applications must be submitted to: FMHS REC Administrator– FMHS-ResearchEthics@nottingham.ac.uk **at least two weeks before the meeting.** This is necessary to ensure that all members of the Committee can adequately review the application. Applications received after this time will be held over until the following meeting. Approval cannot be given to protocols by Chair's action.*

*Except Studies involving blood taking only, where the information generated is of no prognostic significance (i.e. for lab based cell biology studies) These studies will be approved by Chair's action provided no more than a total of 500mls of blood is taken over a 6-month period and no more than 200mls is taken on a single occasion. For these studies applicants need to submit 1 copy of this form and the other required documentation plus a cover letter detailing the nature of the study.

15. AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made

List details of all protocol amendments here whenever a new version of an approved document is produced
This is not necessary prior to initial REC submission