Research Protocol

Sodium intake effect on Aldosterone Level in real Time (SALT)

IRAS Project ID: 354064

University of BRISTOL

Table of Contents

1. Study summary

Hypertension is a major cause of both mortality and morbidity in the UK. Oversecretion of the hormone aldosterone is implicated in around 30% of cases of hypertension seen in secondary health care. At present very few of these patients with hyperaldosteronism are diagnosed as the protocols for diagnosis are complex and invasive. In a recent study, we developed a technique for the continuous monitoring of aldosterone using a novel device called U-Rhythm[1] and found that this was able to differentiate abnormal aldosterone secretion in many cases. In order to improve our diagnostic efficacy we need to show whether changing the dietary input of salt can inhibit aldosterone secretion in normal subjects but not in people with hyperaldosteronism. We first need to do a pilot study to see how changes in salt intake alter the 24-hour regulation of aldosterone in normal subjects.

The aim of our study is to assess whether high or low salt diet affects aldosterone levels for a healthy individual over a 24-hour period.

Our proposed project would involve recruiting 12 healthy volunteers and asking them to adhere to a high or low salt diet for 7 days before using the U-Rhythm technique to monitor aldosterone (and additional hormones) for 24 hours. We will also collect a 24-hour urine sample to measure sodium and aldosterone levels, and blood tests for renin and aldosterone (lying and standing) and renal function (including electrolytes). Additionally, we will ask the participants to exercise on a static bike as aldosterone has been shown to change after exertion[2,3]. After a washout period the process will be repeated following 7 days adhering to the alternate diet. All dietary regimens have been prepared by NHS dietitians and will be balanced in all other nutritional components.

2. Sponsor details

University of Bristol

Adam Taylor, Head of Research Governance, University of Bristol.

Division of Research, Enterprise and Innovation, 1 Cathedral Square, Bristol BS1 5DD. Tel: +44(0)1173940177. Email: research-governance@bristol.ac.uk

3. Chief investigator and research team

Chief Investigator

Dr Emma C. Hart, Ph.D. Senior Lecturer in Cardiovascular Physiology, School of Physiology, Pharmacology & Neuroscience, Biomedical Sciences Building, University of Bristol, Bristol, BS8 1TD. Tel (mob): 07703786721, Fax: 44-(0)1173311472. Email: emma.hart@bristol.ac.uk

Co-Investigators

Dr Tim Swinn, Academic Clinical Fellow, School of Physiology, Pharmacology & Neuroscience, Faculty of Biomedical Sciences, University of Bristol, Bristol, BS8 1TD. Tel: 01173311971. Email: timothy.swinn@nhs.net

Dr Angus Nightingale, Consultant Cardiologist and Senior Clinical Lecturer, Bristol Heart Institute, University Hospitals Bristol and Weston NHS Foundation Trust, Upper Maudlin Street, Bristol, BS2 8HW. Tel (sec): 01173426572; Tel (direct): 0117 342 6691, and University of Bristol, Bristol, BS8 1TD. Email: angus.nightingale@bristol.ac.uk.

Dr Thomas Upton, Clinical Research Fellow in Automated Sampling, University of Bristol Medical School, Dorothy Hodgkin Building, Lewins Mead, BS1 3NY. Tel: 01173311971. Email: Thomas.upton@bristol.ac.uk

Prof Stafford Lightman, Professor of Medicine, University of Bristol Medical School, Dorothy Hodgkin Building, Lewins Mead, BS1 3NY. Tel: 01173311971. Email: Stafford.lightman@bristol.ac.uk

Research staff

Dr Lydia Simpson, BHF Immediate Post-Doctoral Fellow, School of Physiology, Pharmacology & Neuroscience, Faculty of Biomedical Sciences, University of Bristol, Bristol, BS8 1TD. Tel: 01173311971. Email: Lydia.simpson@bristol.ac.uk

Dr Florence Mouy, NIHR Academic Clinical Fellow, School of Physiology, Pharmacology & Neuroscience, Biomedical Sciences Building, University of Bristol, Bristol, BS8 1TD. Tel: 01173311971. Email: florence.mouy@bristol.ac.uk

Dr Hazel Blythe, Postdoctoral Researcher, School of Physiology, Pharmacology & Neuroscience, Faculty of Biomedical Sciences, University of Bristol, Bristol, BS8 1TD. Tel: 01173311971. Email: hazel.blythe@bristol.ac.uk

4. Background

The hormone aldosterone is implicated in around 30% of cases of hypertension. In normal subjects this hormone is regulated both by the renin-angiotensin system and by ACTH secreted by the pituitary gland[4]. In a recent study[1], continuous monitoring of aldosterone using a novel device called U-Rhythm found that aldosterone follows a variety of patterns amongst healthy volunteers; in some volunteers aldosterone peaked once daily (a diurnal rhythm) while others had multiple peaks per 24 hours (an ultradian rhythm). The diurnal pattern was very similar to that displayed by the hormone cortisol, which suggests aldosterone may be predominantly regulated by ACTH in those volunteers. The ultradian pattern included peaks overnight (when cortisol is low) suggesting alternate regulation, presumably via angiotensin 2.

Salt intake is known to effect plasma renin activity and consequently aldosterone concentration. This was not controlled in the U-Rhythm pilot study. We hypothesise that variation in salt intake may, at least in part, explain the different patterns observed. If the hypothesis is correct, we would expect a high salt diet to lead to a diurnal (ACTH dependent) pattern of aldosterone as we expect salt would reduce the levels of renin and angiotensin 2. Conversely, we would expect a low salt diet to result in greater levels of renin and angiotensin 2 resulting in a non- ACTH dependent ultradian rhythm of aldosterone concentration.

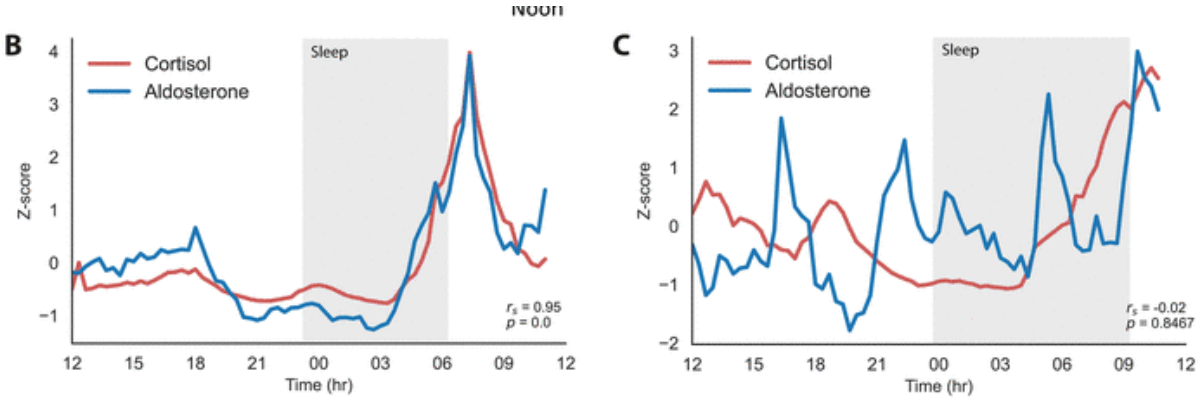

Figure 1: Figure from Upton et al (2003)[1] showing how cortisol and aldosterone varied in 2 healthy volunteers. Note how aldosterone (blue line) follows cortisol (red line) closely in panel B, however there is very little correlation in panel C. We wish to explore whether salt intake may explain the difference in healthy volunteers.

Learning how salt intake affects dynamic changes in aldosterone secretion- especially overnight- may lead to improved methods for diagnosing abnormal patterns of aldosterone levels and thereby allow improved therapy.

5. Aims and hypothesis

5.1 Primary aim

To assess whether altering salt intake for a healthy individual affects their aldosterone profile over a 24-hour period.

5.2 Hypothesis

The high salt diet and low salt diet will have difference aldosterone profiles. Specifically, we expect the high salt diet will lead to a diurnal rhythm (as renin may be suppressed, meaning the dominant regulatory factor is ACTH) and low salt diet an ultradian rhythm (with renin as the primary regulator).

5.3 Null hypothesis

There will be no difference in aldosterone profiles between study arms.

6. Participants and recruitment

6.1 Sample size and power calculation

For this pilot study we will recruit 12 healthy volunteers.

6.2 Inclusion criteria

All participants:

- Aged 18-40 years

6.3 Exclusion criteria:

All participants:

- A history of hypertension, hyperaldosteronism, obstructive sleep apnoea, or heart disease.
- Taking medication that could affect aldosterone levels. This includes most antihypertensives and common inhalers.
- Anyone with a 1st degree family member who was diagnosed with hypertension below the age of 60 or has a diagnosis of primary hyperaldosteronism.
- Inability to understand spoken or written instructions given in English
- Body mass index ≥30 kg/m^2
- Pregnancy

- Alcohol consumption (>28 units/week), daily use of nicotine (including smoking and vaping) and daily use of recreational drugs due to risk of withdrawal symptoms during lab visits
- Needle phobia
- Allergy to any ingredient within meal plans, or dietary requirements that cannot be catered for within the meal plans
- Anyone who has worked shifts finishing after midnight in the past 4 weeks
- Anyone with irregular sleep times (i.e. bedtime/wake time varies by more than +-1 hour across a normal week)

6.4 Recruitment

Participants will be recruited via posters and leaflets displayed around the University of Bristol and public spaces in the wider area (e.g. local shops, cafes, community centres and GP reception areas); study adverts placed in community/University newsletters; study adverts emailed through University Faculty email; information on our research groups website, http://www.uhbristol.nhs.uk/hypertension; and finally social media posts.

Permission will be gained before posters/leaflets are left in public spaces and before adverts are emailed through university email.

Additionally, individuals that have given permission for the research group to store their contact details and to be contacted about taking part in studies may be invited to take part in the study.

7. Study design

This will be an open-label crossover study. We shall recruit 12 volunteers aged 18-40. All subjects will be screened with a general health history, recording of blood pressure, electrocardiogram and blood tests (renal function, electrolytes, and thyroid stimulating hormone).

The subjects will undergo 2 different manipulations of their sodium intake using dietary regimens have been prepared by NHS dietitians and will be balanced in all other nutritional components. The sodium intake in each protocol is listed below:

1) 7 days low salt diet (<400 mg sodium per day)

2) 7 days high salt diet (around 5.9g sodium per day)

Participants will be asked to adhere to both protocols, with at least a week's washout in between. On the final day of each protocol the subject will have 24-hour interstitial

steroid sampling using our U-rhythm system[1] and 24-hour urinary sodium and aldosterone measurement.

Day of study	**Events**	**Location**
Pre-study	Screening questionnaire issued via phonecall and study visits booked if eligible.	Telephone
Day 0	Study visit 1 (1 hour) [labelled visit 4 on 2nd arm of study] - Meet at NIHR Clinical Research Facility - Consent form - BP check, ECG, urinalysis, blood tests (U&E, TSH) - Give meals for the week or arranges time for delivery - VO2 peak test on static bike (to provide reference for exercise test at visits 3 and 6) - Gives 24-hour urine collection bottle - Calibrates and loans Aktiia optical blood pressure monitor - Loans motion-watch or similar	NIHR Clinical Research Facility (CRF), St Michael's Hill
Day 1-6	Participant follows meal plan and keeps sleep diary Contact researcher if any issues (study email address: salt-study@bristol.ac.uk or designated phone number)	
Day 7	Participant starts 24-hour urine collection with 2nd micturition of the day Study visit 2 (1 hour) [labelled visit 5 on 2nd arm of study] - U-Rhythm set-up - Given sleep-recording device (Acu-Pebble or similar) - Given 24-hour diary Participant completes 24-hour diary (recording meal times, sleep/wake times and any exercise undertaken).	NIHR CRF, St Michael's Hill
Day 8	24-hour urine finishes after 1st micturition of the day Study visit 3 (4 hours) [labelled visit 6 on 2nd arm of study] - Weight and blood pressure measured - Participant adopts supine posture on arrival - Post-diet questionnaire - Blood tests (U&E, renin, and aldosterone after 30 mins lying then at 20, 40, and 60 minutes standing plus stored samples for future analysis) - Exercise on static bike (30 minutes at 50-60% VO2 peak) - Serum renin and aldo after exercise - U-rhythm disconnected 2 hours after exercise finish	NIHR CRF, St Michael's Hill

- 24-hour urine sample, U-rhythm, and sleep device returned
- Diet protocol stopped after visit (i.e. after breakfast on day 8) unless there has been any issue with U-rhythm recording, in which was the participant will be asked to extend the study for a further day and conduct a full visit 3 or 6 the next day

If this is the participant's first arm of the study, arrange dates for 2nd arm.

£150 compensation for time given in voucher form after completion of each arm of the study.

7.1 Diversity and Patient and public involvement

Participants will be surveyed at the end of each dietary arm to assess how easy each diet was to adhere to and whether they have any suggestions about the protocol.

We will aim to recruit a diverse range of participants (gender and ethnicity) to reflect the local population in Bristol.

7.2 End of study definition

When last participant has completed final visit and any follow-up monitoring, and data collection is finalised.

8. Outcomes and Statistics

Primary outcome:

- Difference in aldosterone profiles as measured by cosinor analysis of the 24-hour curves and dynamic markers.
- Dynamic markers include: total area under the curve, magnitude of morning peak, number of peaks, or evening nadir.

Secondary outcomes:

- Difference in magnitude of renin or aldosterone response to change in posture
- Difference in magnitude of aldosterone response to exercise
- Difference in 24-hour urine aldosterone

9. Ethical Considerations

9.1 Safety

9.1.1 Screening procedures

The risk associated with conducting screening procedures is identification of incidental findings. Should abnormalities in ECG and blood pressure monitoring and / or pregnancy test identified, the participant will be informed, as they may be unable to participate in the rest of the study. With the participant's permission, their GP will be informed of the findings in writing and the participant will be encouraged to see their GP about the results.

9.1.2 Venepuncture

Risks to the participant of venepuncture include phlebitis, failure to access the vein, thrombophlebitis, bruising and localised pain at the insertion site, clot formation and introduction of flora to site precipitating infection. To minimise these risks, venepuncture will be performed by a doctor or suitably qualified member of the research team. The risk of venepuncture to the qualified professional performing venepuncture is needle-stick injury. This risk will be minimised by following trust guidelines on venepuncture, using the appropriate sharps disposal units in accordance with guidance and by following Trust guidance in the incidence of needle-stick injury. The total blood volume drawn will not exceed 50mL and will not have any haemodynamic consequences to the participant.

9.1.3 Microdialysis catheter insertion

This study will present minimal risk to participants. The microdialysis procedure has previously been conducted in hundreds of participants with no significant adverse events (e.g. ULTRADIAN, REC 16/SW/0069, NCT02934399; REC 47081; REC 77081, [1,5–7]. There is a possibility of minor bruising at the site of the microdialysis probe insertion. The procedure is minimally invasive. A fine catheter is inserted just beneath the surface of the skin using aseptic technique. The catheter remains in place for approximately 24 hours and the risk of infection are considered minimal. The probe, microdialysis pump and accessories used for the subcutaneous tissue collection device are commercially available (CE marked) and no problems are anticipated in relation to these items.

The CGMS sensor is a CE marked device. This is inserted into clean skin of the abdomen or arm by the study investigator (contralateral side to microdialysis probe) following manufacturer recommended standard procedure. This is considered a very low risk procedure.

All other sensors are non-invasive and simply require contact with the skin. There is no anticipated risk associated with these devices.

9.1.4 Ramped exercise tolerance testing

Exercise-induced changes in blood pressure may be experienced by some participants. Either an increase or decrease, during and / or after exercise may occur. A research nurse or doctor will be always present, with continuous ECG and blood pressure monitoring. Any adverse changes will be acted upon. The protocol will be stopped immediately should any participant experience discomfort or distress.

9.1.5 Pregnancy testing

Premenopausal females will be asked to confirm that they are not pregnant and will be offered a pregnancy test prior to participating in the study to confirm they are not pregnant. This test is mandatory. The urine pregnancy test will be completed on all visit days (before commencing any procedures) by a researcher or research nurse.

9.2 Research Materials

9.2.2 Data

The data collected in this study will be for research purposes only. Data will be stored in linked-anonymised format and collected and kept in accordance with ICH-GCP guidelines in a secure, locked location. Participants will be able to view their results and receive an explanation from the investigator upon request.

9.2.2 Research Equipment

Participants will be asked to take utmost care of equipment loaned during the study however the study team will ultimately be responsible for any damage to equipment. No equipment will be loaned to the NHS site.

9.3 Specimen Handling and Laboratory Procedures

Venous blood will be collected by a research nurse or doctor. Samples will be labelled with Study name and ID number and participant age and sex. Labelling with age and sex facilitates quicker identification of results outside the normal range for the age/sex of the participant. Venous blood to be tested for renal function will be immediately placed in the appropriate sample tube, labelled, and stored on ice within a box. This will be stored within the study room until the study visit is completed at which point, the sample will be delivered by a researcher or research nurse to the UH Bristol and Weston Clinical Biochemistry department where they will be processed immediately. Additional serum samples will be processed at the Biomedical Sciences Building (University of Bristol) and stored there at -70°c for future analysis of hypertension biomarkers. Acellular interstitial fluid and urine samples will be securely sent to the University of Bergen for analysis.

9.4 Informed Consent

Individuals expressing interest in participating in the study will be given a copy of the participant information sheet to read, which details the procedures involved and the associated risks. Those interested in taking part will receive a pre-screening telephone call from a researcher or a research nurse, to rule out obvious exclusion criteria. This is also an opportunity for potential participants to ask questions about the study. Those still interested will then be booked into a visit at the NIHR Clinical Research Facility. At this visit, the procedures and risks will be explained in full to participants, who will then have the opportunity to ask questions. Those wanting to take part will be asked to sign a consent form, a copy of which will be given to the participant.

9.5 Right to Withdraw

Participants will be able to withdraw from the study at any point without providing an explanation. This will be explained to participants in the participant information sheet, on the consent form and verbally prior to the start of the experiment. Withdrawal from the study will not affect the care or legal rights of the participant, or their relationship with the University of Bristol.

9.6 Confidentiality

Participant confidentiality will be maintained using Study ID codes on samples and records.

9.7 Sex/Minority Mix

Males and females of all ethnic groups will be eligible to participate in the study. We will aim to recruit members from the BAME community. Bristol has a large BAME community, and this is represented in our healthcare workforce. We will engage with local representatives of the BAME community through the staff BAME committee in the hospital to identify any concerns and find ways to maximise participation.

10. Reporting incidental findings

It is possible that incidental findings concerning participants' health could be identified during the study, for example abnormality in the ECG, blood pressure or pregnancy test. Should incidental findings be identified, both the participant and their GP will be informed. Permission will be gained before a participant's GP is informed of incidental findings. If the participant does not want their GP informed, only the participant will be informed of any findings; however, a letter will be written to the participant to inform them of the findings.

11. Data handling, confidentiality and disposal

Personalised study data will be maintained at the University of Bristol in paper and/or electronic format. Both paper and electronic records will be kept in a locked cupboard in a locked room in a department with security-limited access. Access to the records is restricted to researchers working on the study. Password protection will be used for electronic data and, for the purposes of data analysis, anonymised data will be held on an encrypted flash drive, to be locked as above when not in use. No identifiable data will be stored on laptop computers or portable electronic devices. Analysis will take place by the study team led by Dr Emma Hart and collaborators (using anonymised data). Data will be collected and retained in accordance with the Data Protection Act 2018. Study documents (paper and electronic) will be retained in a secure location during and after the trial has finished. All source documents will be retained for a period of fifteen years following the end of the study. Where trial related information is documented in the medical records – those records will be identified by a 'Do not destroy before dd/mm/yyyy' label where date is fifteen years after the last patient last visit.

The Chief Investigator, Dr Angus Nightingale, will have control of and act as custodian of the data on behalf of the University of Bristol and University Hospitals Bristol and Weston NHS Foundation Trust.

Personal data will be stored for 15 years at the University of Bristol in electronic and hard copy. Access will be controlled by Dr Angus Nightingale who will continue to act as custodian.

12. Monitoring procedure

12.1 Monitoring and Quality Assurance

Good Clinical Practice (GCP) is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human participants. Compliance with this standard provides public assurance that the rights, safety, and well-being of study participants are protected, consistent with the principles that originated in the Declaration of Helsinki and that the clinical study data are credible. This research study will be run in accordance with GCP.

The University of Bristol monitors 10% of its studies. Monitoring is carried out by University Hospitals Bristol and Weston NHS Foundation Trust under a service level contract.

12.2 Direct Access to Source Data / Documents

The chief investigator will allow monitors persons responsible for the audit, representatives of the Ethics Committee and of the Regulatory Authorities to have direct access to source data / documents. This is reflected in the Participant Information Sheet (PIS). The study will be monitored and audited in accordance with Sponsor procedures and undertaken by University Hospitals Bristol and Weston NHS Foundation Trust R&I team on behalf of the Sponsor.

13. Publication procedure

13.1 Definition of authorship

An author is considered to be someone who has made substantive intellectual contribution to a study. Many journals consider it best practice that everyone who is

listed as an author should have made a substantial, direct, intellectual contribution to the work. Honorary or guest authorship is not acceptable.

13.2 Procedure

The baseline criteria for this research for both authorship and acknowledgments for peer reviewed publications and conference contributions is that:

1. Authors must meet all the following criteria:
   - substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data
   - drafting the article or revising it critically for important intellectual content
   - final approval of the version to be published

2. No-one should be omitted from the authorship list if he/she meets the three criteria in 1 above.

3. Some journals allow authorship of multi-centre projects to be attributed to a group. However, all members of the group who are named as authors must still fully meet the above criteria for authorship in 1 above.

4. Other collaborators or members of the research group who may have contributed to some but not all of the criteria in 1 above will be listed in the Acknowledgments (see 6 below).

5. The individual authors will jointly make decisions about authorship before submitting the manuscript for publication. The lead author, corresponding author or the guarantor must be prepared to explain the presence and order of these individuals to the editor of a journal. Authorship and order of authorship (see 7 below) will be agreed in advance, in the early stages of the research.

6. All contributors who do not meet the criteria for authorship will be listed in an Acknowledgments section. Examples of those who might be acknowledged include:
   - persons who have contributed materially to the paper but whose contributions do not justify authorship. These may be listed under such headings as “participating investigators” and their function or contribution should be described - for example, “served as scientific advisors,” “critically

reviewed the study proposal," or "collected data/material". Because readers may infer their endorsement of the data and conclusions, these persons must give written permission to be acknowledged

- a person who provided purely technical help, provided general comments on the manuscript or writing assistance, or a departmental chair who provided general support
- editors can ask corresponding authors to declare whether they had assistance with study design, data collection, data analysis, or manuscript preparation. Authors should therefore disclose in the Acknowledgements section the identity of any individuals who provided this assistance and any entities that supported the work in the published article
- financial support should also be acknowledged and, if appropriate, the grant identified
- material or logistical support, in particular giving recognition to support provided in developing countries, should always be acknowledged

7. Order of authorship
    - the authors shall decide the order of authorship together. Contributors should discuss authorship issues frankly at the start of the work for each anticipated publication and not wait to raise concerns at submission time
    - authors shall specify in their manuscript a description of the contributions of each author and how they have assigned the order in which they are listed so that readers can interpret their roles correctly
    - the corresponding author or guarantor shall prepare a concise, written description of how the order of authorship was decided
    - examples of authorship order include:
        - descending order of contribution
        - placing the person who took the lead in writing the manuscript or doing the research first and the most experienced contributor in the field last
        - alphabetical
        - random order

8. If an individual leaves the project the question of contribution to publications and authorship should be discussed in advance of their departure to minimise misunderstandings and to agree how this will be managed.

14. Quality control and assurance

14.1 Case report forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF will be recorded. All missing data will be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, "N/D" will be inserted. If the item is not applicable to the individual case, "N/A" will be inserted. All entries will be printed legibly in black ink. If any entry errors are made, to correct such an error, a single straight line will be drawn through the incorrect entry and the correct data entered above it. All such changes will be initialled and dated. A random sample of 20% of CRFs will be checked against the computerised data base for quality purposes. This percentage will be increased if a significant error rate is found.

15. Finance and insurance

15.1 Finance

Application is underway for funding from the Bristol and Weston Hospitals Charity.

15.2 Reimbursement to participants

We will reimburse participants for their travel to the Bristol Royal Infirmary and Clinical Research Facility, Bristol. Participants will receive up to £150 per dietary regimen.

15.3 Insurance

This study will be sponsored by the University of Bristol. The University has Public Liability insurance to cover the liability of the University to research participants. In the event that something goes wrong, and a participant is harmed during the research study there are no special compensation arrangements. If a participant is harmed and this is due to someone's negligence then they may have grounds for a legal action for compensation against Bristol University or the NHS Trust or one of the other parties to the research, but they may have to pay their own legal costs.

16. Study sponsor, Ethics, R&D Approval

The study sponsor is the University of Bristol and approval has been granted by the University of Bristol Research Ethics Committee. HRA approval will be required. This study is not a CTIMP, so MHRA approval is not required. Confirmation of capacity and

capability will be sought from University Hospitals Bristol and Weston NHS Foundation Trust.

17. References

[1] Upton TJ, Zavala E, Methlie P, Kämpe O, Tsagarakis S, Øksnes M, et al. High-resolution daily profiles of tissue adrenal steroids by portable automated collection. Science Translational Medicine 2023;15:eadg8464. https://doi.org/10.1126/scitranslmed.adg8464.
[2] Baffour-Awuah B, Man M, Goessler KF, Cornelissen VA, Dieberg G, Smart NA, et al. Effect of exercise training on the renin-angiotensin-aldosterone system: a meta-analysis. J Hum Hypertens 2024;38:89–101. https://doi.org/10.1038/s41371-023-00872-4.
[3] Buono MJ, Yeager JE. Increases in aldosterone precede those of cortisol during graded exercise. J Sports Med Phys Fitness 1991;31:48–51.
[4] Daidoh H, Morita H, Mune T, Murayama M, Hanafusa J, Ni H, et al. Responses of plasma adrenocortical steroids to low dose ACTH in normal subjects. Clin Endocrinol (Oxf) 1995;43:311–5. https://doi.org/10.1111/j.1365-2265.1995.tb02037.x.
[5] Bhake RC, Leendertz JA, Linthorst ACE, Lightman SL. Automated 24-hours sampling of subcutaneous tissue free cortisol in humans. J Med Eng Technol 2013;37:180–4. https://doi.org/10.3109/03091902.2013.773096.
[6] Bhake RC, Kluckner V, Stassen H, Russell GM, Leendertz J, Stevens K, et al. Continuous Free Cortisol Profiles-Circadian Rhythms in Healthy Men. J Clin Endocrinol Metab 2019;104:5935–47. https://doi.org/10.1210/jc.2019-00449.
[7] Bhake R, Russell GM, Kershaw Y, Stevens K, Zaccardi F, Warburton VEC, et al. Continuous Free Cortisol Profiles in Healthy Men. J Clin Endocrinol Metab 2020;105:dgz002. https://doi.org/10.1210/clinem/dgz002.