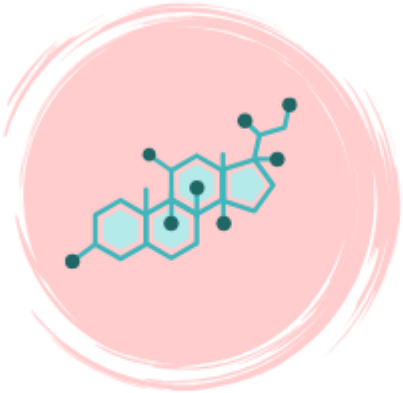
NHS
Health Research Authority

# Comparing the impact of oral and transdermal oestrogen on cortisol binding globulin and cortisol levels before short synacthen test



# OCCS Study

Oestrogen - Cortisol - CBG - Synacthen Study

**Impact of Oral vs. Transdermal Oestrogen on Cortisol & CBG Before SST**

**Oestrogen – cortisol – CBG – Synacthen Study (OCCS Study)**

**Version 4.0 – 10/02/2026**

**RESEARCH REFERENCE NUMBERS**

| | |
|---|---|
| **IRAS Number:** | 356003 |
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| **FUNDERS Number:** | N/A |

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the study publically available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

**For and on behalf of the Study Sponsor:**

Signature:
.....

Date:
...../...../.....

Name (please print):

Julie Dawson

Position:

Research Services Manger

**Chief Investigator:**

Signature:
.....

Date:
...../...../.....

Name: (please print):

Allison Chipchase

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## KEY STUDY CONTACTS

| | |
|---|---|
| Chief Investigator | Dr Allison Chipchase<br>Consultant Clinical Biochemist<br>Norfolk and Norwich University Hospital<br>allison.chipchase@nnuh.nhs.uk<br>01603 287945 (x3945) |
| Lead/student researcher | Mr Rupen Limachya<br>Trainee Clinical Scientist/biochemist<br>Norfolk and Norwich University Hospital<br>rupen.limachya@nnuh.nhs.uk |
| Sponsor | Norfolk and Norwich University Hospitals NHS Foundation Trust R&D Department<br><br>Mrs Julie Dawson<br>Research Operations Office (NNUH)<br>Quadram Institute, Rosalind Franklin Road<br>Norwich, NR4 7UQ<br><br>rdoffice@nnuh.nhs.uk |
| Funder(s) | Norfolk and Norwich Hospitals Charity<br>and<br>Abbott laboratories |
| Key Protocol Contributors | Dr Francesca Swords (NNUH)<br>Consultant in Diabetes and Endocrinology<br>Norfolk and Norwich University Hospital<br>francesca.swords@nnuh.nhs.uk<br>01603 287757 (x3757)<br><br>Dr Rupa Ahluwalia (NNUH)<br>Consultant in Diabetes and Endocrinology<br>Norfolk and Norwich University Hospital<br>rupa.ahluwalia@nnuh.nhs.uk<br>01603 286769 (x2769) |

## STUDY SUMMARY

| | |
|---|---|
| Study Title | Comparing the impact of oral and transdermal oestrogen on cortisol binding globulin and cortisol levels before short synacthen test |
| Internal ref. no. (or short title) | Impact of Oral vs. Transdermal Oestrogen on Cortisol & CBG Before SST |
| Study Design | Prospective, observational, longitudinal cohort study with weekly follow-up over six weeks. Participants are grouped based on oestrogen type (oral, transdermal, or none) and monitored after discontinuation of oestrogen therapy. |
| Study Participants | Adult females aged 18–90 years who are scheduled to undergo a Short Synacthen Test (SST), and are currently on oral or transdermal oestrogen therapy, or not on oestrogen (control). |
| Planned Size of Sample (if applicable) | 72 participants total: 24 per group (oral oestrogen, transdermal oestrogen, control) |
| Follow up duration (if applicable) | 6 weeks of follow-up per participant |
| Planned Study Period | May 2025 – February 2026 |
| Research Question/Aim(s) | **Primary Aim:**<br><br>Can we find out exactly how long women on oestrogen pills need to stop taking them before having a Short Synacthen Test (SST)?<br><br>**Secondary Aims:**<br><br>1. Does stopping oestrogen gel or patches have any effect on test results?<br><br>• There are studies published suggesting that women using transdermal oestrogen do not need to stop treatment before an SST, but there isn't much direct research proving this. This study will confirm whether stopping transdermal oestrogen affects cortisol levels.<br><br>2. Is measuring salivary cortisol (from saliva) a useful and accurate way to assess adrenal function?<br><br>• Blood tests measure both active and inactive cortisol, which can sometimes give misleading results. Salivary cortisol only measures active cortisol, so we will check if it gives a clearer and more accurate picture of adrenal function. |

| | |
|---|---|
| | 3. Do some women's hormone levels take longer to return to normal than others?<br>• We will see if age, type of oestrogen, or other factors affect how quickly CBG and cortisol levels return to normal after stopping oestrogen. |

## FUNDING AND SUPPORT IN KIND

| FUNDER(S) | FINANCIAL AND NON-FINANCIAL SUPPORT GIVEN |
|---|---|
| Norfolk and Norwich University Hospitals NHS Foundation Trust (NNUH) | Non-financial support only: Use of clinical facilities, staff time for screening and recruitment (e.g. endocrinologists), phlebotomy and sample processing services, and R&D governance oversight. |
| Norfolk and Norwich University Hospitals charity | Financial support for cost of travel and parking for participants (£3,450 - £50 per participant) |
| Abbott Laboratories | Financial support for testing |

## ROLE OF STUDY SPONSOR AND FUNDER

The Sponsor for this study is the Research and Development (R&D) Department at Norfolk and Norwich University Hospitals NHS Foundation Trust (NNUH). The Sponsor assumes overall responsibility for ensuring that the study is designed, managed, and conducted in accordance with applicable ethical, legal, and regulatory standards.

There is no external funder for this study. The research forms part of the main/student researcher's MSc project under the NHS Scientist Training Programme (Blood Sciences – Clinical Biochemistry).

### Sponsor Involvement

The Sponsor has:

- Provided oversight of the study design, ensuring it meets ethical and governance requirements;
- Reviewed the protocol prior to submission for HRA and REC approvals;
- Ensured that appropriate indemnity and compliance arrangements are in place.

The Sponsor does not have control over:

- Data analysis or interpretation;
- Manuscript or report writing;
- Final decisions on publication or dissemination of results.

These responsibilities rest with the Chief Investigator (Dr. Allison Chipchase) and the main/student researcher (Rupen Limachya).

## ROLES AND RESPONSIBILITIES OF STUDY MANAGEMENT COMMITEES/GROUPS & INDIVIDUALS

This study does not involve any formal Study Steering Committees, trial Management Groups, or Independent Oversight Committees, as it is a single-centre, student-led project conducted within the NHS and forming part of a postgraduate qualification under the NHS Scientist Training Programme.

### Study Oversight and Coordination

The following individuals will lead study management and oversight:

- **Chief Investigator – Dr. Allison Chipchase**

  Provides clinical and regulatory oversight of the study, reviews study progress, and is accountable for protocol compliance and governance.

- **Main/Student Researcher – Mr. Rupen Limachya**

  Responsible for the day-to-day management of the study, including participant recruitment, data collection, documentation, and reporting.

- **Clinical Supervisors – Dr. Rupa Ahluwalia and Dr. Francesca Swords**

  Provide clinical support in participant screening and recruitment, and contribute clinical guidance during the conduct of the study.

These individuals form the informal project team, which will meet regularly to discuss progress, challenges, and any necessary changes to study delivery.

### Patient & Public Involvement (PPI)

No formal Patient and Public Involvement (PPI) group was established for this study due to its scale and student-led nature. However, the research question was directly informed by ongoing patient experiences and feedback observed in clinical practice, particularly around the inconvenience and symptom burden caused by the six-week oestrogen withdrawal before SST.

## PROTOCOL CONTRIBUTORS

### Sponsor and Research Team Roles

The Sponsor for this study is the Research and Development (R&D) Department at Norfolk and Norwich University Hospitals NHS Foundation Trust (NNUH). The Sponsor is responsible for ensuring that appropriate systems are in place to manage and monitor the study in compliance with ethical, legal, and regulatory standards.

The following individuals have contributed to the development and conduct of this study:

- **Dr. Allison Chipchase** – *Chief Investigator and Project Supervisor*

  Provides overall leadership and oversight of the study, including protocol development, regulatory compliance, and supervision of the student researcher. Dr. Chipchase will also support data interpretation and final reporting.

- **Mr. Rupen Limachya** – *Main/Student Researcher*

  Led the design and drafting of the protocol, and is responsible for coordinating all aspects of the study, including data collection, participant communication, analysis, and preparation of the Master's dissertation and any resulting publications.

- **Dr. Rupa Ahluwalia** and **Dr. Francesca Swords** – *Clinical Supervisors (Consultants in Endocrinology, NNUH)*

  Provided expert clinical input into the study design and will support the screening and recruitment of eligible participants. Their input has been key in aligning the protocol with routine clinical care practices and ensuring the study's feasibility within the NHS setting.

### Sponsor Oversight and Publication Rights

The NNUH R&D Department, as Sponsor, oversees the governance and compliance aspects of the study but will not influence data analysis, interpretation, or publication decisions. The Chief Investigator and main/student researcher retain full control over all dissemination activities.

No external funders are involved in this project.

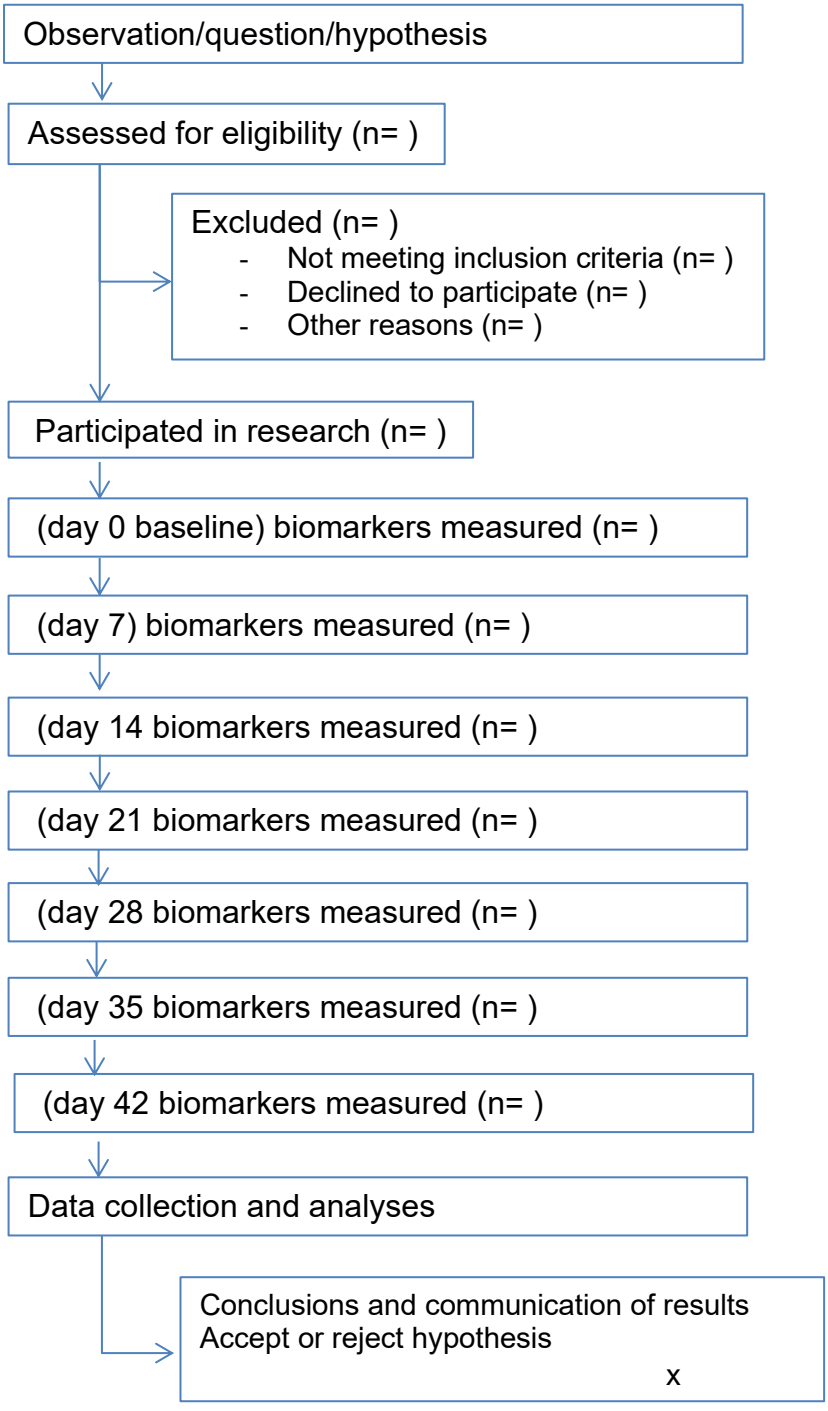
### Patient and Public Involvement

Formal patient and public involvement (PPI) was not undertaken during protocol development due to the study's student-led nature. However, the research question was informed by patient concerns frequently raised in clinic about the burdens of oestrogen withdrawal and testing delays. Participants will be given the option to receive a lay summary of the study results after completion.

**KEY WORDS:**

Oestrogen
Cortisol
Cortisol binding globulin (CBG)
Synatchen test
Contraceptive
Transdermal
Oestradiol

**STUDY FLOW CHART:**



**STUDY PROTOCOL**

Comparing the impact of oral and transdermal oestrogen on cortisol binding globulin and cortisol levels before short synacthen test.

## 1 BACKGROUND

The hypothalamic-pituitary-adrenal (HPA) axis is a critical neuroendocrine system that regulates the body's response to stress, immune function, metabolism, and homeostasis. It functions through a cascade: the hypothalamus secretes corticotropin-releasing hormone (CRH), which stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH). ACTH then acts on the adrenal cortex to produce and release cortisol, the body's main glucocorticoid hormone. Dysfunction at any level of this axis can result in adrenal insufficiency (AI), a condition characterised by inadequate cortisol production. The SST is the most widely used dynamic test to evaluate adrenal function. It involves administering synthetic ACTH (Synacthen) and measuring serum cortisol levels before and after the injection to assess adrenal responsiveness. The SST is primarily used to diagnose primary adrenal insufficiency (where the adrenal glands are directly affected) and secondary or tertiary adrenal insufficiency, which results from impaired ACTH release due to pituitary or hypothalamic dysfunction. Accurate interpretation of the SST is essential, as misdiagnosis can lead to either unnecessary lifelong steroid treatment or missed cases of adrenal failure, both of which carry serious health implications. However, exogenous oestrogen therapy, particularly in oral form, can affect cortisol measurements, potentially confounding the results of the SST.

Cortisol in circulation is largely bound to cortisol-binding globulin (CBG), a glycoprotein produced by the liver. Only a small fraction of cortisol is free (biologically active). Oestrogen is known to increase hepatic production of CBG, thereby increasing total cortisol levels, while free cortisol may remain unchanged. Immunoassays, which are commonly used in clinical laboratories, measure total serum cortisol, both bound and free and do not distinguish between the two. This introduces a problem: individuals on oestrogen therapy may present with falsely elevated cortisol levels, potentially masking adrenal insufficiency.

### Oestrogen Route Matters

The route of oestrogen administration significantly influences the impact on CBG levels. Oral oestrogen undergoes first-pass hepatic metabolism, leading to increased hepatic synthesis of CBG. In contrast, transdermal oestrogen, such as gels and patches, bypasses the hepatic first-pass effect and is therefore considered to have minimal impact on CBG levels; hence, it has been suggested that a 6-week break may not be necessary for transdermal users.

Qureshi et al. (2007) demonstrated that women on oral oestrogen therapy had significantly elevated CBG and total cortisol levels compared to those on transdermal therapy or no oestrogen at all. This finding was supported by Shifren et al. (2008), who also observed that oral conjugated equine oestrogens increased hepatic proteins, including CBG, more than transdermal preparations. As such, guidelines, including those from the Society for Endocrinology and local NHS protocols, often recommend that women on oral oestrogen discontinue therapy for six weeks before an SST to allow CBG levels to return to baseline. At NNUH, currently, both oral and transdermal oestrogen users are required to take a 6-week break.

While this guidance is widely followed, the evidence base for the six-week cessation period is limited, and the impact of shorter withdrawal periods has not been well-studied. Additionally, requiring women to stop oestrogen for six weeks may have significant implications for symptom management,

particularly in postmenopausal women and those on hormone replacement therapy (HRT) for long-term health reasons. Withdrawal from oestrogen can lead to the return of menopausal symptoms such as hot flushes, night sweats, mood swings, fatigue, vaginal dryness, sleep disturbances, and joint pain. For some women, these symptoms can be distressing and affect their quality of life. Shortening this break, even by one or two weeks, could improve patient experience, compliance, and streamline diagnostic pathways.

**Salivary Cortisol – A Promising Alternative**

Another dimension to this issue is the type of cortisol assay used. Most current SST interpretations rely on total serum cortisol, measured by immunoassays. However, as mentioned, this includes cortisol bound to CBG, making the results susceptible to CBG alterations. Salivary cortisol, in contrast, reflects only the free, unbound fraction, which is biologically active and unaffected by changes in CBG. It has been increasingly recognised as a valuable alternative in dynamic endocrine testing.

A recent study by Bäcklund et al. (2024) found that salivary cortisol and cortisone measurements provided a more accurate assessment of adrenal function in women on oral contraceptives, avoiding the false reassurance caused by elevated total serum cortisol. Their findings support the broader adoption of salivary cortisol as a reliable, non-invasive marker, particularly in cases where serum cortisol may be misleading.

While salivary cortisol testing has already been validated in other contexts, such as the diagnosis of Cushing's syndrome and late-night cortisol assessments, its use in SST interpretation remains under investigation. If proven effective, salivary cortisol could reduce the need to adjust hormone replacement therapy and improve patient comfort and safety.

## 2 RATIONALE

Though the impact of the oestrogen route on cortisol dynamics is acknowledged, there remains a gap in prospective, structured data examining how cortisol and CBG levels change week-by-week after stopping oestrogen and whether the 6-week withdrawal period can be safely shortened. Understanding the timeline of CBG and cortisol normalisation is essential for tailoring clinical guidance and reducing unnecessary disruption to HRT. Furthermore, while it has been shown that transdermal oestrogen does not require cessation before SST, more evidence would strengthen this recommendation and provide reassurance for both clinicians and patients. Real-world studies that reflect routine practice and patient variation can help confirm and consolidate existing literature. More evidence is also needed to support the routine use of salivary cortisol and cortisone in adrenal testing, particularly in women on oestrogen therapy. While these markers reflect the biologically active hormone levels and are unaffected by CBG, their measurement, especially by LC-MS, remains technically demanding and less accessible. This study aims to provide additional data on their reliability and practicality in the context of the Short Synacthen Test.

This study aims to address these evidence gaps through a prospective, observational design involving 72 women, divided into three groups: those on oral oestrogen, transdermal oestrogen, and a control group not on oestrogen therapy, who will discontinue their oestrogen therapy for 6 weeks. All participants will undergo an SST at week 6 as part of their routine clinical care. Participants in the oral and transdermal groups will discontinue their oestrogen therapy at week 0, and will undergo weekly blood tests for serum cortisol and CBG, along with weekly salivary samples for cortisol and cortisone.

This approach allows for tracking the dynamic hormonal changes during withdrawal and determining how quickly CBG and cortisol levels return to baseline, especially in oral oestrogen users.

## 3 THEORETICAL FRAMEWORK

This study is based on clinical and physiological evidence that oestrogen therapy, particularly oral forms, increases CBG levels via first-pass liver metabolism, which can falsely raise total serum cortisol measurements and interfere with the Short Synacthen Test (SST) - a key diagnostic tool used to assess adrenal function. Current practice at NNUH recommends stopping oestrogen, both oral and transdermal, for six weeks before SST, but this timeframe is not well evidenced, creating a clinical gap in how best to prepare patients for accurate testing.

The proposed approach is a prospective, observational study that measures weekly changes in serum cortisol and CBG after stopping oestrogen. Participants will also undergo Short Synacthen Tests at baseline and week 6. This approach allows us to monitor when CBG and cortisol levels return to normal, potentially identifying a shorter, evidence-based withdrawal period. In parallel, salivary cortisol and cortisone will be measured weekly, as these are not affected by CBG and may provide a more accurate and less invasive diagnostic alternative.

This design is suitable because it:

- Provides real-time, longitudinal data on hormone changes after oestrogen withdrawal,
- Directly examines the duration of oestrogen's effect on SST reliability,
- Explores the clinical utility of salivary cortisol, supporting the potential for simplified testing in future care pathways.

The study is framed around established concepts in:

- Endocrinology (cortisol regulation and adrenal function),
- Pharmacokinetics (first-pass metabolism and protein binding),
- Diagnostic science (test accuracy and interpretation),

as supported by key literature such as Qureshi et al. (2007) and Shifren et al. (2008).

# 4 RESEARCH QUESTION/AIM(S)

## Aims

**Primary Aim:** To assess whether the six-week cessation period for oral and transdermal oestrogen prior to SST is necessary or whether a shorter break would be sufficient to restore test accuracy.

**Secondary Aims:**

1. To provide further evidence supporting a continued use of transdermal oestrogen during SST without interruption.

2. To evaluate the potential of salivary cortisol and cortisone as non-invasive alternatives to serum cortisol in SST interpretation, particularly in women affected by oestrogen-related changes in CBG.

## 4.1 Objectives

**Null Hypothesis ($H_0$):** Serum cortisol and cortisol-binding globulin (CBG) levels do not significantly change within six weeks of cessation of oestrogen, and SST results remain affected throughout the period.

**Alternative Hypothesis** ($H_1$): Serum cortisol and CBG levels significantly change following cessation of oestrogen, so SST results are no longer affected before the end of the six-week period, indicating that a shorter withdrawal time may be sufficient.

## 4.2 Outcome

The broad outcomes of this study are to determine whether the current six-week withdrawal period for oral and transdermal oestrogen before Short Synacthen Testing (SST) is clinically necessary, or whether a shorter discontinuation period would still allow for accurate test results.

The study also aims to generate further prospective evidence supporting a continued use of transdermal oestrogen without interruption prior to SST, in line with current assumptions, but based on direct observational data.

In addition, the study seeks to explore whether salivary cortisol and cortisone could offer a reliable, non-invasive alternative to serum cortisol, which may lead to improved patient comfort, reduced need for blood tests, and greater accessibility of adrenal testing.

These outcomes will inform clinical guidelines and potentially support more flexible, patient-friendly hormone testing protocols, particularly for women on hormone replacement therapy.

## 5 STUDY DESIGN and METHODS of DATA COLLECTION AND DATA ANALYIS

### Study Design

This is a prospective, observational, longitudinal cohort study involving 72 women aged 18–90 years. Participants are assigned to one of three groups based on their current hormone status: oral oestrogen, transdermal oestrogen, or no oestrogen. All participants will undergo routine clinical SST at Week 6. This design allows real-time tracking of biochemical changes after oestrogen withdrawal and supports evaluation of salivary biomarkers as non-invasive alternatives.

### Methods of Data Collection

Data will be collected through the following:

- **Serum blood samples:** 5–7 mL drawn weekly using SST tubes at 08:00–10:00 to measure serum cortisol, CBG and oestradiol. Additional serum samples will be collected at 0, 30 and 60 minutes during SSTs (Week 6).
- **Saliva samples:** 1 mL using Salivette® or passive drool, collected weekly at the same time as blood samples to measure cortisol and cortisone via LC-MS/MS.
- **Demographic and clinical data:** including oestrogen use history, medical background, and basic anthropometric data, will be collected at baseline via a case report form.
- All sample appointments will be scheduled with participants at enrolment and held weekly at consistent times to minimise diurnal variation.

Sample and data collection will be carried out by the research team and trained NHS phlebotomy staff (sample collection only), with samples stored and processed by the hospital laboratory. The primary researcher will oversee all sample logging, labelling, and chain-of-custody documentation.

### Data Analysis

- Serum and salivary hormone levels will be entered into a secure, anonymised database using participant codes.
- Data will be analysed using statistical software (e.g. SPSS or R).
- Repeated measures ANOVA and regression modelling will assess changes in hormone levels over time.
- Normalisation of cortisol and CBG will be defined using baseline values, weekly measurements and comparison with Week 6 SST results.
- Salivary cortisol and cortisone levels will be compared to serum cortisol values to assess correlation and diagnostic utility.

### Data Handling and Confidentiality

- All data will be pseudonymised at collection.
- Any identifiable data (e.g. contact info, NHS number) will be stored separately in encrypted files.
- Data will be transcribed and coded by the primary researcher.
- Access will be limited to the study team and monitors.

- Final data will be archived securely at Norfolk and Norwich University Hospitals NHS Foundation Trust, with access controlled by the Sponsor. Study data and documentation will be stored for 5 years as per the NNUH Archiving SOP.

## 6 STUDY SETTING

This is a single-centre study that will take place at Norfolk and Norwich University Hospitals NHS Foundation Trust (NNUH). All participant recruitment, consent, data collection, sample collection, and analysis will be carried out at this site. Additional facilities, due to pressures on the NHS, may be used; one such facility is the Quadram Institute (Address**:** Research Park, Rosalind Franklin Rd, Norwich NR4 7UQ). This facility may be used for blood sample collection only.

### Where and How Participants Are Accessed

Participants will be identified from within the hospital's endocrinology outpatient clinics, specifically from patient lists of those already scheduled to undergo a Short Synacthen Test (SST) as part of routine care. Eligibility will be confirmed using clinic referral information, and patients will be approached in person during clinic appointments or contacted by telephone or email. No public advertising will be used.

### Why This Setting Is Appropriate

NNUH is a large NHS teaching hospital with an established endocrine unit and access to a regular flow of eligible patients undergoing SST. This makes it ideally suited to identifying and recruiting women who meet the inclusion criteria. The site has on-site phlebotomy services, a hospital laboratory equipped for sample processing.

### Activities by Site

Study activities, including participant identification, recruitment, consent, blood and saliva collection, SST administration, and sample processing, will take place at NNUH, with the exception of blood sample collection, which may be collected at the Quadram Institute.

### Site-Specific Requirements

The site must support:

- Access to early morning blood draw appointments (08:00 –10:00) for cortisol sampling,
- Phlebotomy staff for weekly sampling,
- Secure sample storage (-20°C or -80°C),
- Laboratory space for serum separation and storage,
- LC-MS access for salivary cortisol and cortisone analysis (if not performed on-site, samples will be sent to a validated lab).

There are no known barriers or limitations at the chosen site that would interfere with delivery of the study.

# 7 SAMPLE AND RECRUITMENT

## 7.1 Eligibility Criteria

- Female participants aged 18–90 years.
- Scheduled for a Short Synacthen Test.
- Able to give informed consent.

### 7.1.1 Inclusion criteria

- Female patients aged 18 to 90 years.
- Scheduled to undergo a Short Synacthen Test (SST) as part of routine clinical care.
- Currently taking oral or transdermal oestrogen therapy for ≥3 months, or not on oestrogen (control group).
- Willing and able to provide informed consent.
- Willing and able to discontinue oestrogen therapy (if applicable) for 6 weeks.
- Willing and able to provide weekly blood and saliva samples over a 6-week period.

### 7.1.2 Exclusion criteria

- Pregnant or breastfeeding women.
- Significant liver dysfunction, defined as ALT or AST >2× the upper limit of normal, or low albumin (<25 g/L).
- Currently taking glucocorticoids above replacement dose or have done so in the past 6 months.
- Recent intra articular steroid injection (within 12 weeks)
- Unable to comply with study procedures or follow-up schedule.
- Individuals who were not assigned female sex at birth
- Women using oestrogen hormone-modulating medications
- Chronic kidney disease affecting hormone metabolism
- Diagnosed with adrenal insufficiency or any known pituitary or adrenal disorder that could affect cortisol production.
- Any endocrine disorder (e.g., Cushing's syndrome, uncontrolled thyroid disease) that could interfere with the study results
- Use of drugs that significantly alter liver metabolism (e.g., anticonvulsants, rifampicin), as they may impact CBG levels.

## 7.2 Sampling

Once a patient has been enrolled for the trial, the following samples will be taken at the designated time points (day 0, day 7 (week 1), day 14 (week 2), day 21 (week 3), day 28 (week 4), day 35 (week 5), day 42 (week 6).

Take the following blood samples:

- **Blood (serum** cortisol, CBG and oestrogen**):** 5–7 mL in (yellow top) tube, collected 08:00 – 10:00, weekly for 6 weeks + during SST at 0, 30 and 60 minutes.
- **Saliva (cortisol & cortisone):** 1 mL using Salivette® or passive drool, collected 08:00 – 10:00, weekly for 6 weeks.
-
- **SST samples:** Additional serum samples at 0, 30 and 60min during SST (Week 6), 5 mL each in SST tube.

### 7.2.1 Size of sample

Sample size: 72 participants – 24 per group with 3 groups

Due to limited data in the literature and the novel aspect of measuring CBG, serum and salivary cortisol over 6 week period, which has not been done before. We used mean cortisol for baseline and mean cortisol levels for someone who is on oral oestrogen (this can vary drastically based on many factors, including individualised dosage). In addition, we assumed a constant negative slope to work out a difference (may not be representative of how CBG and cortisol actually drop back to baseline over the 6 weeks, but it was an appropriate assumption for the statistical calculation). So, the calculation is based on the mean difference and the standard error of the mean (SD/sqrt(n)). The power calculation came out as 19 per group - rounded to 20 with a power of 0.908.

### 7.2.2 Sampling technique

This study will use a purposive sampling strategy. Participants will be selected based on specific inclusion criteria: women aged 18–90 who are scheduled to undergo a Short Synacthen Test (SST) as part of their routine clinical care and are either currently taking oral oestrogen, transdermal oestrogen, or no oestrogen (control group).

**Where the Sample Is Derived From**

The sample will be drawn from patients attending the endocrinology outpatient clinics at Norfolk and Norwich University Hospitals NHS Foundation Trust. Eligible participants will be identified through clinic records, referral pathways, and SST booking lists.

**Rationale for Sampling Strategy**

Purposive sampling is appropriate given the study's focus on specific subgroups of women (oral vs. transdermal vs. no oestrogen). This approach aligns with the study's theoretical framework, which is based on the known pharmacological effects of different oestrogen delivery routes on cortisol-binding

globulin (CBG) and cortisol levels. Random sampling is not appropriate, as the study requires participants with predefined hormone exposure profiles to compare biochemical outcomes over time.

This strategy ensures that the sample includes participants who are most relevant to the research question, allowing the study to effectively explore the impact of oestrogen type and withdrawal duration on the accuracy of the SST and the utility of salivary cortisol.

### 7.3 Recruitment

The direct care team (e.g. endocrinologists, specialist nurses, or clinic staff) using clinic lists, SST appointment schedules, or electronic health records will identify potential participants. Only patients already scheduled for an SST as part of their routine clinical care will be considered. Once identified, eligible patients will be approached either in person during clinic appointments or by phone/email/letter and provided with a Participant Information Sheet. The research team, in collaboration with the clinical team, will conduct screening for eligibility. Follow-up calls after posting/emailing will help ensure recruitment and initial verbal consent to attend the first blood sample collection, where written consent is then obtained.

#### 7.3.1 Sample identification

**Who Will Identify Participants and What Method Will Be Used?**

Members of the direct clinical care team at Norfolk and Norwich University Hospitals NHS Foundation Trust will identify participants. This includes endocrinologists, endocrine specialist nurses, or other authorised staff involved in the patient's care. These team members will use the hospital's electronic health record systems, including ICE (Integrated Clinical Environment), to review relevant clinic and referral information and identify patients scheduled for Short Synacthen Tests who meet the inclusion criteria.

**Resources Used**

- ICE and other hospital electronic health record systems will be used to access clinic referrals, laboratory orders, and recent medical history.
- A secure screening log will be maintained by the research team to record potentially eligible participants.
- Only members of the clinical team will access identifiable data for initial screening.

**Recruitment Pathway**

A member of the clinical team will make the first approach to potential participants, either in person at a clinic appointment or by telephone/email/letter, with the addition of a Participant Information Sheet, followed by a call by the clinical team or research team to address any questions, and arrange for consent.

**Recruitment Centres and Publicity**

- This is a single-centre study, with all recruitment taking place at Norfolk and Norwich University Hospitals.
- No Patient Identification Centres (PICs) will be used.

- Participants will not be recruited via posters, social media, public adverts, or external websites.

**Use of Identifiable Personal Information**

The patient's clinical team will only access identifiable information to assess eligibility and make initial contact. The research team will only access identifiable data after informed consent has been given.

**Referral and Communication**

Referrals to the research team from the clinical team will be made securely. All potential participants will receive a Participant Information Sheet (PIS) and be given sufficient time to consider their decision before participating.

**Participant Payments**

Participants will be reimbursed for reasonable travel expenses for visits outside of their usual care. No other payments or incentives will be offered.

### 7.3.2 Consent

**Consent Process Overview**

Informed consent will be obtained before any study-specific procedures take place. Only individuals capable of giving informed consent for themselves will be invited to participate.

**Who Will Take Consent**

Consent will be taken either by me, the student/main researcher, Rupen Limachya, by one of the endocrinologist doctors/consultants involved in the participant's care, the senior endocrine specialist nurse or anyone outlined as part of the research/study team. All individuals involved in the consent process will be fully trained and listed on the study delegation log. If Rupen Limachya is taking consent, this will only occur after initial identification and approach by the clinical team or in their presence.

**Consent Procedure**

- Eligible participants will be identified by the clinical care team and approached in person or via telephone/email.
- They will be provided with a Participant Information Sheet (PIS) and Informed Consent Form (ICF), both of which have been approved by the Research Ethics Committee (REC).
- Participants will be given sufficient time to review the documents, ask questions, and discuss with others if they wish.
- The researcher or consultant will confirm the participant:
    - Understands the purpose, procedures, risks, and voluntary nature of the study
    - Has the capacity to make an informed decision
    - Knows that they can withdraw at any time without affecting their care
- One copy of the signed consent form will be given to the participant, one retained in the Investigator Site File, and one may be stored in the patient's medical notes.

### Assessing Capacity

Capacity will be assessed informally by the person taking consent. Only participants who are able to understand, retain, and weigh the information, and communicate their decision, will be enrolled in the study.

### Voluntariness and Safeguards

Participants will be made aware that participation is entirely voluntary, and their decision will not affect their NHS care or treatment. They may withdraw at any point without giving a reason.

### 7.3.3. Definition of End of Study

The trial will end when the last participant completes their final visit, including the week 6 Short Synacthen Test (SST) and all associated sample collections (serum, salivary cortisol, CBG, oestradiol).

This definition is appropriate as it marks the completion of all primary and secondary outcome data collection, with no further participant involvement required

## 8 ETHICAL AND REGULATORY CONSIDERATIONS

This study has been designed to meet all ethical and regulatory requirements for conducting research involving human participants within the NHS. The research question: evaluating whether the six-week withdrawal period for oral oestrogen prior to SST can be shortened, addresses a clinically relevant issue that has direct implications for patient care, comfort, and diagnostic accuracy.

### Risk and Benefit Balance

The study poses minimal risk to participants. The primary risk is the temporary return of menopausal symptoms such as hot flushes, mood changes, and poor sleep following the withdrawal of oral or transdermal oestrogen. These symptoms are expected to resolve once the participant resumes their usual treatment. There is also minor discomfort associated with weekly blood sampling, though this is common in clinical care.

There is no direct benefit to participants, but the results of this study could significantly improve care for future patients, particularly those undergoing SST while on hormone replacement therapy. It may lead to shorter treatment interruptions and the use of simpler saliva-based tests, reducing the burden on both patients and the healthcare system.

### Respect for Participant Dignity

All interactions with participants will be handled sensitively and respectfully, with clear communication, privacy during sample collection, and ongoing opportunities to ask questions or withdraw. The use of saliva samples, which are non-invasive and painless, reflects the study's commitment to minimising participant burden.

**Data Protection and Confidentiality**

All personal data will be pseudonymised, stored securely, and handled in accordance with the UK GDPR and Data Protection Act 2018. Participants will be informed about how their data will be used, stored, and protected. Only authorised research personnel will have access to identifiable data, and all data processing will comply with local NHS trust policies.

**Legal and Regulatory Compliance**

This research will be conducted in accordance with:

- The UK Policy Framework for Health and Social Care Research
- Good Clinical Practice (GCP) principles
- Health Research Authority (HRA) guidance
- Research Ethics Committee (REC) approval
- Local R&D approval at Norfolk and Norwich University Hospitals NHS Foundation Trust

All procedures and materials (including consent forms and participant information sheets) will be submitted for HRA and REC approval before recruitment begins.

## 8.1 Assessment and management of risk

**Study Risks**

This study involves minimal physical or psychological risk to participants. The primary risks relate to:

- Withdrawal of oestrogen therapy for six weeks may result in the temporary return of menopausal symptoms such as hot flushes, mood changes, tiredness, and poor sleep.
- Weekly blood draws carry a minor risk of discomfort, bruising, or bleeding.

These risks will be discussed with participants in advance, and support will be offered throughout the study.

**Safeguarding and Risk of Disclosure**

Although the study does not involve interviews or detailed psychological assessments, there is still a remote possibility that a participant could disclose information suggesting:

- Risk of harm to themselves
- Risk of harm to others

If such a situation arises during any interaction with the research team:

1. The participant's immediate safety will be prioritised.
2. The researcher will escalate the concern immediately to a member of the participant's clinical care team, usually the consultant endocrinologist, or to an appropriate NHS safeguarding lead, in line with local trust safeguarding policy.
3. If the participant is at immediate risk of harm, emergency procedures will be followed - this may include contacting crisis services or emergency medical staff.

4. Any disclosures that involve potential harm to others (e.g. violence or abuse) will also be escalated to the safeguarding team or other relevant authorities, in line with NHS safeguarding protocols.

**Safeguarding Governance**

- Rupen Limachya has completed NHS safeguarding training and will follow Norfolk and Norwich University Hospitals NHS Foundation Trust safeguarding policy.
- A named clinical supervisor (consultant endocrinologist) will be available to advise on all safeguarding concerns.
- All disclosures and actions taken will be documented and stored securely.

This plan ensures that any potential risk to participants or others is identified and managed quickly, ethically, and safely.

### 8.2 Research Ethics Committee (REC) and other Regulatory review & reports

Before the study begins, a favourable ethical opinion will be sought from a Research Ethics Committee (REC) via the Health Research Authority (HRA). This review will include the study protocol, informed consent forms, participant information sheet, and other supporting documents.

As the study is being conducted within the NHS, it will be submitted through the NHS Research Ethics Service, in line with the UK Policy Framework for Health and Social Care Research.

Regulatory Responsibilities

- Substantial amendments requiring REC review will not be implemented until a favourable opinion is received and local approval mechanisms are in place.
- All correspondence with the REC will be retained and stored securely.
- The Chief Investigator, Allison Chipchase (Consultant Clinical Scientist and Principal Investigator), is responsible for:

    - Submitting Annual Progress Reports (APR) to the REC within 30 days of the anniversary of the favourable opinion, and annually thereafter.
    - Notifying the REC when the study has ended.
    - Submitting a final report within one year of study closure, including study results and any publications or abstracts.
    - Immediately notifying the REC if the study is ended prematurely, with a clear explanation of the reasons.

The main/student researcher, Rupen Limachya, will assist with these processes under the Chief Investigator's supervision, ensuring all requirements are fulfilled in line with regulatory standards and GCP.

## Regulatory Review & Compliance

Before the study begins at Norfolk and Norwich University Hospitals NHS Foundation Trust, the Chief Investigator, Allison Chipchase (Consultant Clinical Scientist), or her designee, Rupen Limachya (main/student researcher), will ensure that all necessary regulatory and local approvals are in place. This includes obtaining confirmation of capacity and capability from the Trust's R&D department, following the standard approval process outlined by the Health Research Authority (HRA) for NHS research in England.

This is a single-site NHS study, and no non-NHS organisations will be involved.

## Amendments

Should any amendments to the study be required - including changes to the protocol, participant documents, or study procedures, these will be submitted to the appropriate regulatory bodies (e.g. REC or HRA) by the Chief Investigator or, main researcher, with prior agreement from the study Sponsor.

Once regulatory approval for an amendment is received, the Chief Investigator or designee will work with the R&D department and other relevant hospital staff to ensure all necessary arrangements are in place before the amendment is implemented at the site.

All communications, submissions, and approvals related to regulatory compliance will be documented and retained in the Study Master File, in accordance with Good Clinical Practice (GCP) and institutional policies.

## Amendments

This study will be conducted within the NHS and therefore follows the Health Research Authority (HRA) and Research Ethics Committee (REC) guidelines on managing amendments.

Process for Making Amendments

If changes to the study are needed (e.g. modifications to the protocol, informed consent documents, sample schedules, or study procedures), these will be reviewed and prepared by the Chief Investigator, Allison Chipchase, in collaboration with the main/student researcher, Rupen Limachya. Any proposed amendment will first be reviewed in agreement with the Sponsor.

It is the Sponsor's responsibility to determine whether an amendment is substantial or non-substantial.

- Substantial amendments (those that may impact participant safety, data integrity, or study conduct) will be submitted to the REC via a valid Notice of Amendment.
- The REC will respond within 35 calendar days, and the amendment will only be implemented once a favourable opinion is received.
- Non-substantial amendments, such as administrative changes, will be documented and communicated to the local R&D office and stored in the Study Master File.

If relevant, other review bodies (e.g. Confidentiality Advisory Group) will also be notified, in accordance with their guidance.

**Communication of Amendments**

All approved substantial amendments will be:

- Communicated to the Norfolk and Norwich University Hospitals NHS Foundation Trust R&D department,
- Shared with the local research delivery team,
- Reflected in updated versions of participant-facing documents (e.g. consent forms, information sheets),
- Registered in the Study Amendment Log, with clear version control.

The Chief Investigator will ensure that the most up-to-date versions of all documents are in use at the site and that all stakeholders are aware of the changes before implementation.

**Tracking Amendment History**

All amendments, whether substantial or not, will be recorded in a central amendment tracking log. Each change will be version-controlled and dated, ensuring that the most current version of the protocol and associated study documents is clearly identifiable.

## 8.3 Peer review

This study has undergone internal peer review as part of the approval process within the Norfolk and Norwich University Hospitals NHS Foundation Trust and through the University of Manchester as part of the Scientist Training Programme (STP). Senior academic and clinical supervisors with expertise in endocrinology and clinical research methodology reviewed the protocol.

As this is a single-site, student-led study forming part of a formal research training programme, the level of peer review is considered proportionate to the size, scope, and complexity of the study. The review assessed both the clinical relevance of the research and the robustness of the methodology.

## 8.4 Patient & Public Involvement

This study has been developed with consideration of published guidance on hormone withdrawal, cortisol testing, and diagnostic challenges, but has not included formal patient or public involvement (PPI) in the design or development stages due to its nature as a single-site, student-led training project forming part of the NHS Scientist Training Programme (STP).

However, the study addresses a research question that arose from routine clinical practice and patient concerns, specifically regarding the burden of stopping oestrogen therapy for extended periods before undergoing adrenal function testing. These concerns have been frequently voiced by patients attending the endocrine clinic at Norfolk and Norwich University Hospitals NHS Foundation Trust, particularly regarding the return of menopausal symptoms and delays in testing and diagnosis.

**Future Involvement**

Although formal PPI was not undertaken in the early stages, patients will be consulted at the dissemination phase, with the option to provide feedback on how they would like to access study

results. A summary of the findings will be made available to all participants, and future iterations of the project or related studies may incorporate structured patient involvement.

The study team recognises the value of PPI and will consider incorporating patient input in future work or follow-up studies where feasible.

### 8.5 Protocol compliance

All staff involved in the study will be expected to adhere strictly to the approved study protocol, as well as to Good Clinical Practice (GCP) and local research governance policies.

Protocol Deviations and Non-Compliance

It is acknowledged that accidental protocol deviations may occasionally occur. These will be:

- Immediately reported to the Chief Investigator, Allison Chipchase, and the Sponsor,
- Documented appropriately using the relevant deviation reporting forms,
- Reviewed to determine whether corrective or preventive action is needed.

The study team will investigate repeated or systematic deviations. Recurring non-compliance may be classified as a serious breach of the protocol and escalated in accordance with Sponsor SOPs and HRA guidance.

All deviations, breaches, and their management will be recorded in the protocol deviation log, stored securely in the Study Master File.

The main/student researcher, Rupen Limachya, will be responsible for supporting compliance monitoring, under the oversight of the Chief Investigator. Any deviations identified will be addressed promptly to ensure the integrity of the study and the safety of participants.

### 8.6 Data protection and patient confidentiality

All investigators and study staff involved in this research will comply with the UK General Data Protection Regulation (UK GDPR) and the Data Protection Act 2018 in the collection, storage, processing, and disclosure of personal information. The principles of confidentiality and data minimisation will be upheld at all times.

**Data Collection and Pseudonymisation**

Participants will be assigned a unique study ID, and all research samples and data will be labelled using this code. No directly identifiable information will appear on any study materials used for analysis. A separate, securely stored linking file will connect participant identifiers to their study IDs, accessible only to authorised personnel.

**Data Security**

- Identifiable personal data (such as names and NHS numbers) will be stored in encrypted and password-protected files on secure NHS servers.

- The linking file will be kept separately from the research dataset and accessible only by the Chief Investigator and main/student researcher.

**Confidentiality and Limited Access**

Access to identifiable information will be strictly limited to personnel who require it for study administration, quality control, or oversight. All staff handling such data will be trained in confidentiality procedures and data protection requirements.

**Data Transmission**

Only pseudonymised data will be transferred or shared, and it will be done via secure NHS-approved systems. Identifiable data will not be shared with anyone outside the research team.

Data Retention and Custodianship

- Personal identifiable data will be retained only until the end of the study, at which point it will be securely destroyed in line with NHS Trust policies.
- The research dataset (de-identified) may be retained longer for analysis and audit, if required by the Sponsor.
- The Chief Investigator, Allison Chipchase, will act as the Data Custodian for the study and will ensure compliance with all data protection requirements.

## 8.7 Indemnity

Norfolk and Norwich University Hospitals NHS Foundation Trust, which will provide the necessary indemnity arrangements in accordance with NHS indemnity schemes and UK legal frameworks, sponsor this study.

**Sponsor Indemnity for Management of the Research**

Norfolk and Norwich University Hospitals NHS Foundation Trust, as the Sponsor, holds NHS indemnity to meet its potential legal liability for harm to participants arising from the management of the research.

**Sponsor Indemnity for Design of the Research**

As the Sponsor, Norfolk and Norwich University Hospitals NHS Foundation Trust will also provide indemnity to cover its legal liability for harm arising from any flaws or deficiencies in the design of the research.

**Indemnity for Investigators and Site Personnel**

This study is being conducted entirely within the NHS at a single site. All investigators and site staff involved in the conduct of the study are covered by NHS professional indemnity under the terms of

their employment at Norfolk and Norwich University Hospitals NHS Foundation Trust. No non-NHS staff or sites are involved.

**Compensation for Harm Where No Legal Liability Arises**

The Sponsor does not routinely offer compensation where no legal liability arises. However, participants will be informed that they retain the right to pursue compensation through the legal system in the event of harm.

**Insurance for Study Equipment**

No special or external equipment will be provided to the site for this study. All materials used (e.g. phlebotomy and sample collection kits) are standard NHS equipment and will be used in accordance with existing hospital procedures. Therefore, no additional equipment-related insurance or indemnity is required.

## 8.8 Access to the final study dataset

Access to the final, full study dataset will be limited to the following individuals:

- Chief Investigator: Allison Chipchase
- Main/Student Researcher: Rupen Limachya
- Authorised members of the Norfolk and Norwich University Hospitals NHS Foundation Trust R&D and data governance teams, if required for audit or compliance purposes.

This is a single-centre study, so no other sites or investigators will require access to the full dataset, and there is no steering group in place.

The final dataset will be used to support:

- The completion of a Master's project dissertation as part of the NHS Scientist Training Programme (STP), and
- The preparation of a peer-reviewed publication, where appropriate.

The dataset will be securely stored in a pseudonymised format. No identifiable participant data will be used in the dissertation or any publication. All study participants will be informed of this potential use in the Participant Information Sheet and Informed Consent Form.

No additional or secondary analyses will be undertaken without further ethical approval and appropriate consent.

# 9 DISSEMINIATION POLICY

## 9.1 Dissemination policy

### Ownership and Use of Study Data

The data arising from this study will be owned by the Sponsor, Norfolk and Norwich University Hospitals NHS Foundation Trust. The main/student researcher (Rupen Limachya) will have access to the dataset for the purpose of conducting analysis and preparing outputs for their Master's research project and dissertation as part of the MSc Blood Sciences (Clinical Biochemistry) within the Scientist Training Programme (STP).

### Final Study Report

At the conclusion of the study, the collected data will be:

- Analysed and tabulated,
- Used to produce a Final Study Report, and
- Submitted as part of the student's MSc dissertation.

### Publication and Authorship

The main/student researcher may prepare a peer-reviewed scientific publication based on the study findings. The Chief Investigator (Allison Chipchase) and other relevant contributors may be listed as co-authors in line with contribution and authorship guidelines.

There are no embargoes or time restrictions placed on publication by the Sponsor. All publications will include appropriate acknowledgement of the Sponsor and any supporting staff or services.

### Participant Notification

Participants will be offered the option to receive a lay summary of the study results after data analysis is complete. This will be shared via email or post, depending on the participant's preference.

### Requests for Individual Results

The study does not produce individual clinical results relevant to patient care. However, participants can request a summary of the overall study results from the research team if they consented to receiving study results.

### Public Availability of Study Materials

There are no current plans to publish the full dataset, protocol, or statistical code in a public repository. However, anonymised data or methodologies may be shared within future publications, in line with ethical approvals and data governance requirements.

## 9.2 Authorship eligibility guidelines and any intended use of professional writers

### Authorship Criteria

Authorship on the final study report and any resulting publications will follow the International Committee of Medical Journal Editors (ICMJE) authorship guidelines. Individuals must contribute significantly to:

1. The design or execution of the study, or the collection, analysis, or interpretation of data;
2. Drafting or revising the manuscript critically for important intellectual content;
3. Final approval of the version to be published;
4. Accountability for all aspects of the work, ensuring accuracy and integrity.

### Anticipated Authors

The following contributors are currently expected to meet authorship criteria:

- Mr Rupen Limachya – Main/Student Researcher
- Dr Allison Chipchase – Chief Investigator and Supervisor
- Dr Francesca Swords – Consultant in Diabetes and Endocrinology (NNUH)
- Dr Rupa Ahluwalia – Consultant in Diabetes and Endocrinology (NNUH)
- Dr Trevor Tam – Foundation year 2 doctor
- Ms Neetha Joseph – Specialist endocrinology nurse
- Dr Thazin Wynn - speciality registrar for endocrinology

Others who provide support (e.g. administrative, clinical, or technical) but do not meet all authorship criteria will be acknowledged in the final report or publication.

### Use of Professional Writers

No commercial or professional medical writers will be used in producing the final report or any manuscripts.

## 10 REFERENCES

Bäcklund, N., Lundstedt, S., Tornevi, A., Wihlbäck, A. C., Olsson, T., Dahlqvist, P. and Brattsand, G. (2024) 'Salivary Cortisol and Cortisone Can Circumvent Confounding Effects of Oral Contraceptives in the Short Synacthen Test', *J Clin Endocrinol Metab,* 109(7), pp. 1899-1906.

Goodman, M. P. (2012) 'Are all estrogens created equal? A review of oral vs. transdermal therapy', *J Womens Health (Larchmt),* 21(2), pp. 161-9.

Qureshi, A. C., Bahri, A., Breen, L. A., Barnes, S. C., Powrie, J. K., Thomas, S. M. and Carroll, P. V. (2007) 'The influence of the route of oestrogen administration on serum levels of cortisol-binding globulin and total cortisol', *Clin Endocrinol (Oxf),* 66(5), pp. 632-5.

Shifren, J. L., Rifai, N., Desindes, S., McIlwain, M., Doros, G. and Mazer, N. A. (2008) 'A comparison of the short-term effects of oral conjugated equine estrogens versus transdermal estradiol on C-reactive protein, other serum markers of inflammation, and other hepatic proteins in naturally menopausal women', *J Clin Endocrinol Metab,* 93(5), pp. 1702-10.

Verbeeten, K. C. and Ahmet, A. H. (2018) 'The role of corticosteroid-binding globulin in the evaluation of adrenal insufficiency', *J Pediatr Endocrinol Metab,* 31(2), pp. 107-115.

# 11. APPENDICIES

## 11.1 Appendix 1- Required documentation

The following documentation will be required prior to study initiation at the research site (Norfolk and Norwich University Hospitals NHS Foundation Trust):

- Signed and dated CVs of all members of the research team, including Chief Investigator (Dr. Allison Chipchase) and main/student researcher (Mr. Rupen Limachya)
- Good Clinical Practice (GCP) training certificates for all staff involved in consent and study procedures
- Final approved version of the Study Protocol
- Final approved Participant Information Sheet (PIS) on NHS headed paper
- Final approved Informed Consent Form (ICF) on NHS headed paper
- Completed and approved IRAS application and supporting documents
- HRA and REC favourable opinion letters
- Confirmation of capacity and capability from the host site's R&D department
- Delegation and Study Responsibilities Log
- Screening and enrolment logs
- Sample tracking forms or case report forms (CRFs)
- Study-specific risk assessment (if applicable)

Additional documents may be included if requested by the site R&D office or Sponsor.

## 11.2 Appendix 2 – Schedule of Procedures

| **Procedures** | **Visits** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Screening** | **Baseline** | **Week 1** | **Week 2** | **Week 3** | **Week 4** | **Week 5** | **Week 6** |
| Informed consent | **x** | | | | | | | |
| Demographics | **x** | | | | | | | |
| Medical history | **x** | | | | | | | |
| Eligibility check | **x** | | | | | | | |
| Short Synacthen Test (SST) | | | | | | | | **x** |
| Blood sample – CBG and serum cortisol | | **x** | **x** | **x** | **x** | **x** | **x** | |
| Salivary sample – Cortisol and cortisone | | **x** | **x** | **x** | **x** | **x** | **x** | **x** |

### 13.3 Appendix 3 – Amendment History

| Amendment No. | Protocol version no. | Date issued | Author(s) of changes | Details of changes made |
|---|---|---|---|---|
| 0 | Version. 0.1 | 24/04/25 | Rupen Limachya | Initial version of protocol submitted for REC and HRA review |
| 1 | Version. 0.2 | 21/07/25 | Rupen Limachya | Changes made to number of participants/participants per group (60 to 72 and 20 per group to 24 per group)<br><br>Removal of all references to finger-prick testing<br><br>Changes to wording associated with transdermal oestrogen withdrawal – not as optional as previously thought.<br><br>Changing sampling time from 08:00 - 09:00 to 08:00 – 10:00<br><br>Changes made to age range from 18-60 to now 18-70 years old |
| 2 | Version. 0.3 | 22/10/25 | Rupen Limachya | Initial SST at baseline/week 0 is now removed from all sectioons due to waiting list pressure at CIU<br><br>Added Dr Trevor Tam, Neetha Joseph and Dr Thazin Wynn as contributors to this study and to the relevant sections<br><br>Added Quadram Institute as an additional site for blood sample collection only<br><br>Added Abbott Laboratories as a financial sponsor<br><br>Removed marking for baseline SST on the Appendix 2 – Schedule of Procedures table and added a marking on blood sample instead.<br><br>Added participants will also be contacted/made aware of the study via |

| | | | | |
|---|---|---|---|---|
| | | | | post/letter with PIS and there will be a follow up call after initial post/letter<br><br>In the exclusion criteria:<br><br>Removed 'known or suspected bone metabolism disorders' – not relevant for this study<br><br>Added a numerical value for low albumin cut off (<25 g/L) – help make recruitment decisions easier<br><br>Changed 'women using OTHER hormone-modulating medications' to 'women using OESTROGEN hormone-modulating medications' – Using 'other' makes it to too broad and therefore difficult to recruit even if the medications has no implications or affects on the study. |
| 3 | 0.4 | 10/02/2026 | Rupen Limachya | Changes made to age range from 18-70 to now 18-90 years old |
| | | | | |