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NOTTINGHAM CLINICAL TRIALS UNIT
at the University of Nottingham

TRIAL PROTOCOL

POISE

Premature **O**varian **I**nsufficiency **S**tudy of **E**ffectiveness of hormonal therapy (POISE)
(Hormone therapy for premature ovarian insufficiency: randomised trial and long-term evaluation)

Protocol version number and date: Final Version 4.0, 01 Oct 2024

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Protocol development and sign off

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Protocol Amendments				
The following amendments and/or administrative changes have been made to this protocol since the implementation of the first approved version				
Amendment number	Date of amendment	Protocol version number	Type of amendment	Summary of amendment
AM09 (SA03)	01 Oct 2024	V4.0	Substantial	Sample size has been adjusted to 380 women. Follow-up period has been reduced to a minimum of 2 years. Inclusion of blood pressure and weight data collection from local GP practices and pharmacies to allow greater flexibility when participants are unable to get to their face to face follow-up appointments. Voucher incentives will now be given to all participants when clinical outcome data is received. A sub-section on

				testosterone has been included under treatment contraindications for clarity.
AM06 (SA02)	16 Jan 2023	V3.0	Substantial	Minor wording changes in inclusion/exclusion to allow clarity. Requirement of washout out with testosterone treatment has been included. Guidance on completing DEXA scans on the same machine has been included.
AM03 (SA01)	28 Jan 2022	V2.0	Substantial	Clarification of sub-study samples and tests. Correction of typographical errors. Staffing change updates. Addition of "previous oestrogen treatment (Yes/No)" as a minimisation variable.

Protocol Approval / Sign-off

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and any subsequent amendments of the clinical trial regulations, GCP guidelines, the Sponsor's (and any other relevant) SOPs, and other regulatory requirements as amended.

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 clinical investigation without the prior written consent of the Sponsor.

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

CI Signature Page	
This protocol has been approved by:	
Trial Name:	Hormone therapy for premature ovarian insufficiency: randomised trial and long-term evaluation
Protocol Version Number:	Version: Final 4.0
Protocol Version Date:	01-Oct-2024 (dd-mmm-yyyy)
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Trial Summary

Full title	Hormone therapy for premature ovarian insufficiency (POI): randomised trial and long-term evaluation
Trial design	Multi-centre prospective, open, two-group parallel randomised controlled, superiority trial with equal allocation (1:1) to receive either hormone replacement therapy (HRT) or the combined oral contraceptive pill (COC).
Objectives	**Primary objective** In women with POI what is the relative effectiveness, in terms of bone mineral density (BMD), of HRT (any route) compared to the COC? **Secondary objectives** 1. To assess the relative effectiveness, of HRT (any route) compared to the COC on menopausal symptoms, quality of life, sexual functioning, weight and blood pressure. 2. To assess the effect of treatment with HRT (any route) compared to COC on adherence with treatment, pregnancy outcome, side effects and serious adverse events. 3. To assess the relative effectiveness of oral and transdermal HRT, compared to the COC in terms of BMD, blood pressure and menopausal symptoms. 4. To compare the prevalence of bone fractures, diagnosis of cardiovascular disease, cancer, cognitive impairment, and mortality between women treated with HRT (any route) and those treated with COC. **Secondary objectives – sub-study** 1. What is the effect of HRT compared with the COC on bone and cardiovascular (CVD) biomarkers, in a subset of women from selected clinics?
Eligibility criteria	**Inclusion Criteria** 1. Diagnosis of POI (based on NICE guidelines) or with established diagnosis of POI (e.g. Turner Syndrome, surgical menopause) 2. Will be aged ≥ 18years up to <40 years at randomisation 3. Not intending to become pregnant within 12 months 4. Not taken any HRT, COC or testosterone treatment for the last 4 weeks or willing to stop HRT/COC/testosterone treatment for a minimum period of 4 weeks prior to randomisation. 5. Must provide written/electronic informed consent **Exclusion Criteria** 1. Contraindications to HRT or COC 2. Taking other drugs affecting BMD e.g. bisphosphonates, and long-term use of systemic corticosteroids (dietary supplements e.g. Vitamin D, calcium and short course of corticosteroids are permitted) 3. Receiving estrogens for puberty induction 4. Participation in a clinical research study (currently or in the last 3 months) involving testosterone treatments or novel HRT formulations

Description of treatment/ interventions	**COC**: 30µg ethinyloestradiol with progestogen, as an extended regimen (tricycling for 63 days followed by 4-7 day interval, or flexible extended use, taking continuously with breaks of 3-4 days if breakthrough bleeding occurs). **HRT**: continuous oestradiol, either oral or transdermal, dose 2mg orally, or 50µg patch or 1.5mg gel transdermally. Women who have not had a hysterectomy will also be prescribed progestogen or progesterone, either oral, intrauterine or transdermal, continuous or cyclical.
Outcome measures	**Primary** The primary outcome is the absolute BMD (g/cm^2) at 2 years from the date of randomisation, assessed by a standard dual energy X-ray absorptiometry (DEXA) scan, of the lumbar spine. **Secondary** The secondary outcomes are detailed below. • Absolute BMD (g/cm^2) in lumbar spine at 1 and 2 years. • Absolute BMD (g/cm^2) in hip at 1 and 2years. • T-score category (≤-2.5, > -2.5 to ≤ -1, > -1) for BMD at lumbar spine at 1, 2 and 5 years. • T-score category for BMD at hip at 1and 2 years. • Individual domains (vasomotor, psychosocial, physical and sexual) and summary score of Menopause Specific Quality of Life (MENQOL-I)-Intervention questionnaire[1] at 3, 6 and 12 months then annually. • Sexual function (pleasure, discomfort and frequency) measured by the Sexual Activity Questionnaire (SAQ)[2] at 3, 6, 12 months then annually. • Work Productivity (absenteeism, presenteeism, work productivity loss and activity impairment), using the Work Productivity and Activity Impairment (WPAI) Scale (Specific health Problem)[3] at 3, 6, 12 months then annually. • Weight measured at 3, 6 and 12 months then annually • Blood pressure (BP) at 3, 6 and 12 months and then annually. • Pregnancy and outcome at 3, 6 and 12 months then annually. • Satisfaction with treatment, on a 5-point Likert scale at 3, 6 and 12 months then annually. • Change or cessation of treatment at 3, 6 and 12 months then annually. • Adverse events at 3, 6 and 12 months and then annually. • Diagnosis of cancer, cardiovascular disease, cognitive impairment, bone fractures and mortality up to 5 years (collected from routine data sources). Sub-study (subset of participants at selected sites) • Bone metabolism markers, liver function tests, bone profile, and 1,25 Vitamin D. Blood and/or urine samples will be collected in a subset of participants from selected sites, at 3 and 12 months. • Cardiovascular markers. Fasting lipids, fasting glucose, HBA1c, insulin like growth factor, and renal function. Blood samples will be collected in a subset of participants from selected sites, at 3 and 12 months and then annually.

Sample Size	The original sample size required to detect a mean difference in lumbar spine BMD (g/cm^2) of 0.05 between the HRT and the COC groups at 2 years post-randomisation using a standard deviation of 0.15, with 90% power, two-tailed significance of 5%, 1:1 allocation, and allowing for 20% loss to follow-up was 480. At a blinded review after 18 months of recruitment, the sample size was adjusted for the correlation between repeated measures and revised to 304, with a target of 380 participants to allow for 20% loss of primary outcome data.
Expected recruitment duration	57 months
Randomisation and blinding	Eligible women will be individually randomised on a 1:1 ratio, minimised by recruitment site, age at randomisation, body mass index, smoking status and previous oestrogen treatment, and retaining a random element, to HRT or the COC treatment groups. Allocation will be concealed using a secure, web-based minimisation algorithm. Blinding of treatment to investigators and participants is neither practical nor ethical due to the diversity in the range of treatment options, and the different contraceptive effect.
Statistical methods	Analysis of the primary outcome measure (absolute lumbar spine BMD at 2 years from the date of randomisation) will be performed using a mixed effects model using all available follow-up data (1 year, 2 year), adjusted for the baseline BMD score and the minimisation variables, with recruiting site entered as a random effect. The model will include a treatment-by-time interaction to estimate the between group difference at each follow-up time-point with 2 years being the primary treatment comparison. Participants will be analysed according to randomised group regardless of treatment actually received.

Trial Participant Flow Diagram

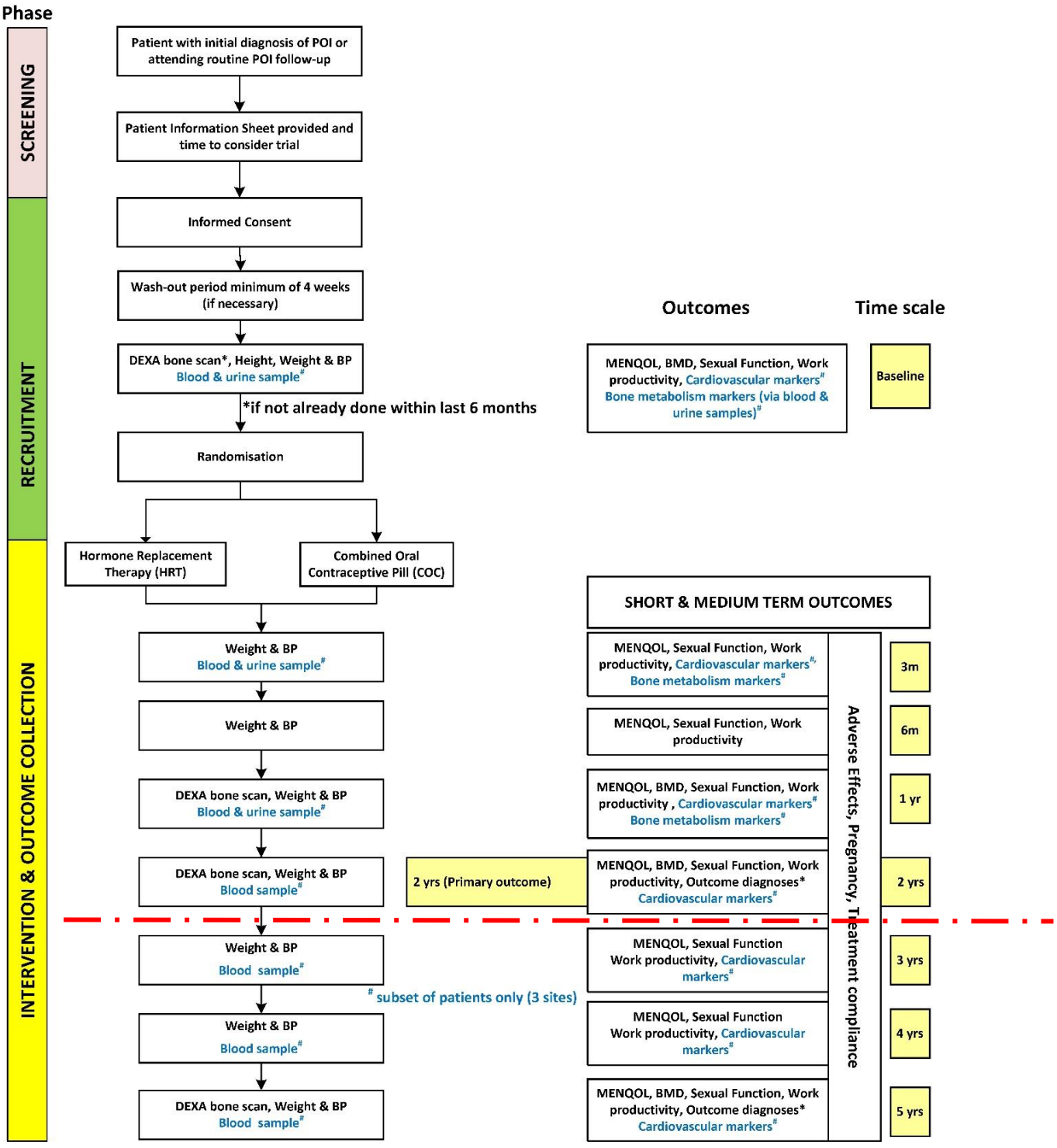

Figure 1: Trial participant flow diagram

*The red line represents that participants will complete up to 5 years, for a minimum duration of 2 years, in the trial

Abbreviations

Term	Description
Abbreviations	
ABPI	Association of the British Pharmaceutical Industry
AE	Adverse Event
AR	Adverse Reaction
BFS	British Fertility Society
BNF	British National Formulary
BMD	Bone mineral density
BP	Blood Pressure
CACE	Complier Average Casual Effect
CI	Chief Investigator
COC	Combined Oral Contraceptive Pill
CONSORT	Consolidated Standards Of Reporting Trials
CVD	Cardiovascular Disease
DEXA	Dual Energy X-ray Absorptiometry
DMC	Data Monitoring Committee
DMP	Data Management Plan
eCRF	Electronic Case Report Form
ESHRE	European Society of Human Reproduction and Embryology
FSH	Follicle-Stimulating Hormone
FSRH	Faculty of Sexual and Reproductive Health
GCP	Good Clinical Practice
GP	General Practitioner
HRT	Hormone replacement therapy
HTA	Health Technology Assessment
ICF	Informed Consent Form
ISF	Investigator Site File
IMP	Investigational Medicinal Product
IVF	*In vitro* fertilisation
LNG-IUS	levonorgestrel-releasing intrauterine system
MedDRA	Medical Dictionary for Regulatory Activities
MENQOL-I	Menopause-Specific Quality of Life (Intervention version)
MHRA	Medicines and Healthcare products Regulatory Agency
NCTU	Nottingham Clinical Trials Unit
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NIHR	National Institute for Health Research
PIC	Participant Identification Centre

PIS	Participant Information Sheet
POI	Premature Ovarian Insufficiency
POISE	Premature Ovarian Insufficiency Study of Effectiveness of hormonal therapy
REC	Research Ethics Committee
RCT	Randomised Clinical Trial
RSI	Reference Safety Information
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SAP	Statistical Analysis Plan
SAQ	Sexual Activity Questionnaire
SD	Standard Deviation
SmPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group
TSC	Trial Steering Committee
UCL	University College London
UAR	Unexpected Adverse Reaction
VTE	Venous thromboembolism
WPAI-SHP	Work Productivity and Activity Impairment Scale (Specific Health Problem)

1. Background and Rationale

1.1. Background

Premature ovarian insufficiency (POI) is a clinical syndrome defined by the loss of ovarian activity before the age of 40 years, which affects at least 1 in 100 women[4]. Although the cause is unknown in most women, it can be familial (in 10%) or associated with autoimmunity or genetic conditions (e.g. Turner syndrome). POI can be a result of treatment (oophorectomy, chemotherapy, or pelvic radiotherapy) and is increasing in young cancer survivors[5].

National Institute for Health and Care Excellence (NICE) guidelines recommend that diagnosis is made by both a history of menopausal symptoms, including amenorrhoea or infrequent periods, and elevated follicle-stimulating hormone (FSH) levels on two blood samples taken 4-6 weeks apart[6]. Other markers of ovarian reserve, such as anti-Mullerian hormone and antral follicle count on transvaginal ultrasound scan, are not currently recommended as diagnostic tests for POI.

POI is associated with low levels of circulating oestrogen, although ovarian function can fluctuate in some women before complete cessation. Symptoms of oestrogen deficiency include hot flushes and night sweats, vaginal dryness causing painful intercourse, low libido, low energy levels, sleep disturbance, lack of concentration, muscle and joint aches and stiffness, skin/hair changes and labile mood.

Long-term outcomes of oestrogen deficiency include loss of BMD, potentially leading to early onset of osteoporosis, fracture and disability, as seen in post-menopausal women[7]; increased risk of cardiovascular disease (CVD) of earlier onset[8], reduced life expectancy[4,9] ; and possibly an increased risk of degenerative neurological disease (Parkinson's disease, cognitive impairment)[10].

Women with POI are provided with advice regarding a healthy lifestyle (involving weight-bearing exercise, avoidance of smoking, maintenance of a normal body weight, and the recommended dietary intake of calcium and vitamin D) for promotion of bone health and reduction of CVD risk. However, lifestyle measures alone are insufficient and all professional bodies recommend long-term systemic hormone treatment at least until the age of natural menopause[6,11] to improve symptoms, to maintain bone health and prevent osteoporosis, and to reduce future CVD risk; topical oestrogen (vaginal pessaries or cream) can be used in addition to treat genito-urinary symptoms.

The two preparations widely prescribed in the UK as oestrogen replacement for women with POI are the combined oral contraceptive pill (COC) and hormone replacement therapy (HRT). These preparations have different oestrogen formulations and may have differing benefits and risks. At present there are no robust data to inform women's decision-making. This results in variations in clinical practice and varying advice that may be confusing. For some women, the choice between the COC or HRT may depend upon contraceptive needs or acceptability of the treatment, but if both are acceptable alternatives, women need to have the information to help make an informed decision.

The COC may be seen as socially acceptable for young women and may reduce the stigma associated with menopause. It is also free of prescription charges in England. The COC, unlike HRT, provides contraceptive cover. Contraception may be desired as 5–10% of women with POI still conceive spontaneously[12]. However, HRT provides physiological replacement of oestrogen and might therefore be a better option for sustaining long-term health. There is some limited evidence to suggest that HRT is superior to the COC in increasing/maintaining bone density, however the strength of this evidence is insufficient to change current clinical practice. Even small differences between treatments may have a large impact on long-term health outcomes, because women with POI are taking hormone

replacement for many years, so it is important to establish definitively the superiority/non-superiority of HRT over the COC.

A 2015 review by NICE identified two small, short-term cross-over randomised clinical trials (RCT) comparing the effects of HRT and the COC, where there was very limited data showing a benefit of HRT on lipid profiles and bone biomarkers, concluding women should be offered the choice and that a long-term RCT was needed[13-15]. A parallel systematic review identified 12 studies of HRT, mostly small, uncontrolled studies with surrogate outcomes and heterogeneity in HRT regimens[16]. A subsequent RCT measured lumbar spine BMD in women with idiopathic POI at two years, finding a significant gain in women taking oral HRT, no change in those on the COC, resulting in a 0.05g/cm^2 (95% CI 0.007 to 0.092, p=0.05) difference between the two groups[17]. This significant BMD improvement was replicated in a retrospective review of 20 women with non-genetic POI, taking oral HRT over 3 years, whilst the 5 women using transdermal HRT showed a non-significant increase and 19 women using the COC saw a decrease[18].

Women with POI have increased risk of osteoporosis and fracture later in life[19,20]. In cross-sectional studies, women with POI have lower BMD than control women and the prevalence of osteoporosis in women with POI is reported in the range of 8-14%[21-23]. Reduced bone density is related to fracture risk, although it is not the only determinant[24]. Fracture occurs at a lower trauma in osteoporotic bone than healthy bone; for each standard deviation the BMD drops below the young adult mean, the risk of sustaining a fracture doubles[25]. The most common fractures associated with osteoporosis are those of the hip, vertebrae and wrist[26]. All are associated with a reduction in quality of life, with a higher impact caused by hip fracture, vertebral fracture or fracture at more than one site[27,28]. Excess mortality rates in the year following a hip fracture, compared with the general population, have been reported as up to 36%; excess mortality persists for several years after the fracture[29]. Fragility fractures are very uncommon in young women, but risk increases with time; in a study of women who had undergone an early surgical menopause, compared with women who had hysterectomy only, the prevalence of fractures at age 70 was significantly higher at 38.9% compared with 23.5%, and BMD was lower[30].

Women with untreated POI have lower bone density than women with POI using hormone replacement[21]. In the setting of a clinical trial, women with POI taking no hormonal treatment lost bone density. This loss in the 'no treatment' group was statistically significant when compared with both the group taking HRT and the group taking the COC, being a mean of 0.052g/cm^2 and 0.013g/cm^2 lower in the lumbar spine, respectively[17].

Women with POI have increased cardiovascular morbidity and mortality[31], and so it is possible that oestrogen replacement may reduce future CVD risk. Although there is a lack of evidence in young women, in 25 POI women using increasing doses of HRT, intima media thickness decreased significantly[32]. Women with Turner syndrome have a high prevalence of hypertension, obesity, glucose intolerance and hyperlipidaemia, so they are at particular risk. A small study in Turner women indicated that HRT improved aortic compliance, fasting glucose and insulin concentrations[33]. A small randomised study of COC versus physiological HRT, using transdermal oestradiol and vaginal progesterone, showed that the latter was associated with lowered blood pressure[14], suggesting that the route of administration might be important.

The European Society of Human Reproduction and Embryology (ESHRE) guideline considers the type and route of HRT[5]. Significant pharmacokinetic differences between oral and transdermal oestradiol exist, the latter more closely represents the physiological state but neither trials nor their results are uniform[34,35]. ESHRE research recommendations state that studies are needed that assess the lifetime

risk of fracture in women with POI, in addition to the effects on life expectancy, quality of life, and neurological function.

The POISE trial aims to answer what is the most effective hormone treatment for women with POI.

1.2. Trial Rationale

1.2.1. Justification for participant population

The target population will be women with a diagnosis of POI, whether idiopathic, autoimmune, chromosomal (e.g. Turner syndrome) or iatrogenic (e.g. following chemotherapy, or surgery such as bilateral salpingo-oophorectomy). POI diagnosis will be based on NICE guidance[6]. Women with established POI (e.g Turner Syndrome or after surgical menopause) do not need repeat investigations (e.g. FSH). The trial will be as inclusive as possible with only women with contraindications to either study drug class, taking other drugs affecting BMD or receiving estrogens for puberty induction being excluded.

Women aged between ≥ 18 years up to <40 years old at the time of randomisation will be included in the trial. The lower age limit of ≥ 18 years is to ensure that where applicable puberty induction has completed (achieving menarche and final height) and the upper age of <40 years is based on the accepted definition of POI[6,36].

Women intending to conceive within the next year will be excluded from entering the trial. Women with POI have impaired fertility and are likely to require assisted conception using *in vitro* fertilisation (IVF) with donor eggs. These procedures have to be planned and are dependent on clinic timescales, donor availability, and funding, meaning that even women who may be starting to consider their options with regards to pregnancy at the point of entering the trial are unlikely to become pregnant within a year. It is hoped by excluding women intending on becoming pregnant within the first year we will maximise the number of participants likely to remain on treatment for POI and for whom primary outcome data can be obtained at 2 years. We would expect any extension of the exclusion period beyond this to further reduce loss of primary outcome and have a negative effect on recruitment with women being less certain of their future fertility plans rendering themselves ineligible.

1.2.2. Justification for design

The POISE trial has been designed to determine if HRT is superior to the COC on important clinical outcomes and patient-reported symptoms, based on the hypothesis that HRT provides more physiological continuous hormone supplementation with natural oestrogens[6].

The outcomes being measured reflect that POI requires long-term treatment and differences in outcomes may not emerge for many months or even years, during which time adherence and outcomes will be affected by confounding factors e.g. pregnancy. Absolute BMD in the lumbar spine has been chosen as the primary outcome, as a survey of 242 patients indicated that osteoporosis was their most important concern, and protection against loss of BMD in the first years following diminishing ovarian activity is associated with a reduced risk of osteoporosis and fractures. Additionally, BMD measurement is accurate and reproducible, less likely to be affected by confounders than other measurements such as cardiovascular markers, easily available in clinical practice, and acceptable to patients.

The trial is powered to detect a small, but clinically significant, difference in the primary outcome at 90% power. It will also have reasonable power to determine small to moderate differences between

the COC and oral HRT, between the COC and transdermal HRT, and between oral HRT and transdermal HRT (non-randomised comparison), dependent upon the proportion of women using HRT by each route.

1.2.3. Choice of treatment

Two preparations are widely prescribed in the UK as oestrogen replacement for women with POI, HRT and COC. HRT is based on physiological oestrogen (17β oestradiol) and usually contains synthetic progestogen although alternatives (natural progesterone, intrauterine progestogen) are available; treatment may be oral or transdermal. The COC contains ethinyloestradiol, a synthetic oestrogen with significant metabolic impact, and synthetic progestogen.

The trial is a two-group design, with participants randomised to receive either HRT or the COC. The type and dose of oestrogen is specified. The type and dose of progestogen is specified, as far as practicable given the preparations available, to standardise the treatments used.

In view of the variety of available hormonal preparations, some with added contraceptive properties, it is neither practical nor ethical to blind the treatments to the women or treating clinicians.

HRT:

The trial mandates that the HRT preparation contains 17β-oestradiol. This is available via oral and transdermal routes, the latter being the preferred choice of half of the clinicians surveyed when designing the trial. However, transdermal HRT may not be as acceptable to women as oral tablets, and patches may cause skin irritation, acknowledged by our patient survey, so the trial will not specify which formulation is used. It is acknowledged that the pharmacodynamics differ. Therefore, it is required that the **oestradiol daily starting dose is 2mg orally or 50µg patch or 1.5mg gel transdermally** (these are considered clinically equivalent). Dose will not be titrated by serum oestradiol measurement as this is not done in clinical practice and of limited value in oral therapy.

Women with POI and an intact uterus require progestogen to protect against endometrial hyperplasia; progestogen may be given cyclically or continuously. This will not be mandated, as the trial design survey indicated that the formulation used was predicated on the need for contraception or other patient preferences, with 15 out of 42 of HRT prescribers surveyed offering the levonorgestrel-releasing intrauterine system (LNG-IUS) and the same number oral micronised progesterone. Women who are not using the LNG-IUS will be informed of the small chance of spontaneous pregnancy in POI and advised to use non-hormonal contraception if required.

The preferred formulations will be specified, but alternatives can be prescribed if required, at the discretion of the treating clinician following discussion with the woman, and adjustments allowed, within the constraints described and with no reduction in oestradiol dose.

COC:

The trial requires participants randomised to receive treatment with COC to commence treatment with a **COC that contains 30µg ethinyloestradiol** in a monophasic formulation. The suggested formulation is 30µg ethinyloestradiol with 150µg levonorgestrel. 30µg has been selected as this is the most commonly prescribed dose in clinical practice and also to limit any impact dose may have on BMD.

Conventional administration of COC is for 21 days followed by a 7-day break to induce a regular withdrawal bleed. However, in January 2019, the Faculty of Sexual and Reproductive Health (FSRH) guidelines[37] were revised to support extended use of monophasic COC, either tricycling for 63 days of

tablets followed by a 4-7 day hormone-free interval, or flexible extended use, taking continuously with short breaks of 3-4 days if breakthrough bleeding occurs. As HRT is taken continuously, the extended COC regimen will be used as the appropriate comparator. The preferred regimen is tricycling for 63 days of tablets followed by a 7-day hormone-free interval.

The contraceptive patch and ring are not widely used, and to reduce the variation in hormone therapy in the COC group, will not be permitted.

1.2.4. Sub-study

To determine the early impact on bone metabolism and cardiovascular health, selected blood and/or urine biomarkers will be assayed from women recruited from at least 3 sites. This will provide insight into the immediate response of bone turnover and contribute to the assessment of cardiovascular effects of hormone treatment. Further details on biomarker collection are provided in Section 8.4.

2. Objectives and Outcome Measures

2.1. Objectives

2.1.1. Primary objective

In women with POI what is the relative effectiveness, in terms of BMD, of HRT (any route) compared to the COC?

2.1.2. Secondary objectives -effectiveness

- To assess the relative effectiveness, of HRT (any route) compared to the COC on menopausal symptoms, quality of life, sexual functioning, weight, and blood pressure.
- To assess the effect of treatment with HRT (any route) compared to COC on adherence with treatment, pregnancy outcome, side effects and serious adverse events.
- To assess the relative effectiveness of oral and transdermal HRT, compared to the COC in terms of BMD, blood pressure and menopausal symptoms.
- To compare the prevalence of bone fractures, diagnosis of cardiovascular disease, cancer, cognitive impairment and mortality between women treated with HRT (any route) and those treated with COC.

2.1.3. Secondary objectives – sub-study

- What is the effect of HRT compared with the COC on bone and CVD biomarkers, in a subset of women from selected clinics?

2.2. Outcome Measures

2.2.1. Primary outcome:

Absolute BMD (g/cm^2) at 2 years from the date of randomisation, assessed by a standard DEXA scan of the lumbar spine.

2.2.2. Secondary outcomes:

The secondary outcomes will be measured at different stages throughout the trial.

- Absolute BMD (g/cm^2) in lumbar spine at 1 and 2 years.
- Absolute BMD (g/cm^2) in hip at 1 and 2 years.
- T-score category (≤-2.5, > -2.5 to ≤ -1, > -1) for BMD at lumbar spine at 1 and 2 years.
- T-score category for BMD at hip at 1 and 2 years.

- Individual domains (vasomotor, psychosocial, physical and sexual) and summary score of Menopause Specific Quality of Life (MENQOL-I)-Intervention questionnaire at 3, 6 and 12 months then annually.
- Sexual function (pleasure, discomfort and frequency) measured by the Sexual Activity Questionnaire (SAQ)[2] at 3, 6, 12 months then annually.
- Work Productivity (absenteeism, presenteeism, work productivity loss and activity impairment), using the Work Productivity and Activity Impairment (WPAI) Scale (Specific health Problem)[3] at 3, 6, 12 months then annually.
- Weight (kg) at 3, 6, and 12 months then annually.
- Systolic blood pressure (mm Hg) at 3, 6 and 12 months then annually.
- Diastolic blood pressure (mm Hg) at 3, 6 and 12 months then annually.
- Pregnancy and pregnancy outcome.
- Satisfaction with treatment, on a 5-point Likert scale at 3, 6 and 12 months then annually.
- Change or cessation of treatment at 3, 6 and 12 months then annually.
- Adverse events at 3, 6 and 12 months and then annually. Specific minor-side effects collected will include breast pain, nausea, headaches, skin or hair changes and unscheduled bleeding (see Section 9.2) as well as more serious events such as venous thromboembolism. All serious adverse events will be collected and causal relationship with treatment considered.
- Diagnosis of cancer, cardiovascular disease, cognitive impairment, bone fractures and mortality will be collected from routine data sources up to 5 years.

Sub-study

- Bone metabolism markers, liver function tests, bone profile, and 1,25 Vitamin D. Blood and urine samples will be collected in a subset of participants from selected sites, at baseline, 3 and 12 months (not reported until the end of the study).
- Cardiovascular markers. Fasting lipids, fasting glucose, HBA1c, insulin like growth factor and, renal function. Blood samples will be collected in a subset of participants from selected sites, at baseline, 3 and 12 months and then annually up to and including 5 years.

3. Trial Design and Setting

3.1. Trial Design

Multi-centre prospective, open, two-group parallel randomised controlled, superiority trial with equal allocation (1:1) to receive either HRT or the COC. Recruitment of 380 women is required to achieve ≥ 90% power to detect a difference in mean of 0.05 g/cm^2 in lumbar spine BMD between HRT and the COC. Further information on sample size and randomisation can be found in Section 6 and Section 13.

Participants will start treatment according to their randomised treatment allocation. Long-term systemic hormone treatment is recommended at least until the age of natural menopause. For the purpose of this trial, participants will be advised to remain on their allocated treatment for a minimum duration of 2 years, however it is acknowledged that a number of factors may influence a participant's choice of therapy over this time.

3.2. Trial Setting

Patients will be recruited from gynaecology clinics (specialist and generalist) in the UK. It is anticipated that around 24 sites will be required to achieve the target sample size required.

Potentially eligible patients are expected to be identified from the following routes:

- Patients attending gynaecology clinics will be approached by a trained member of the site research team about participating in the trial.
- Clinical database searches: Hospital databases/clinic records will be searched by members of the clinical care team to identify patients ahead of their scheduled clinic appointments who have POI and who may be eligible. Patients will be sent a personalised letter offering them the opportunity to potentially take part if they are eligible, along with a copy of the Participant Information Sheet (PIS) and Informed Consent Form (ICF). The letter will include a brief introduction to the study and contact details of the local research team to find out more information and discuss the study further. The letter will also direct them to the trial website where they can find further information.
- Self-referral: Patients may make contact with the research team as a result of trial advertising and promotional material (e.g. posters, leaflets and social media).
- Peripheral hospitals may refer their patients to recruiting sites.
- Endocrinology clinics, clinical genetics and cancer centres may refer patients to the recruiting investigator within their Trust/ Board.
- Additional primary or secondary care settings may be set up as Participant Identification Centres (PICs) if required during the course of the trial. PICs will support recruitment by identifying potential research participants against the trial eligibility criteria and directing potential participants to recruitment sites.

4. Eligibility

4.1. Inclusion Criteria

- Diagnosis of POI (based on NICE guidelines) or with established diagnosis of POI (e.g. Turner Syndrome, surgical menopause)
- Will be aged ≥18 years up to <40 years at randomisation
- Not intending to become pregnant within 12 months
- Not taken any HRT, COC or testosterone treatment for the last 4 weeks or willing to stop HRT/COC/testosterone treatment for a minimum period of 4 weeks prior to randomisation
- Must provide written/electronic informed consent

4.2. Exclusion Criteria

- Contraindications to HRT or COC (guidance available from the British National Formulary and The Faculty of Sexual & Reproductive Healthcare Medical Eligibility Criteria for Contraceptive Use (FSRH UK MEC))
- Taking other drugs affecting BMD e.g. bisphosphonates and long-term use of systemic corticosteroids (dietary supplements e.g. Vitamin D, calcium and short course of corticosteroids are permitted)
- Receiving estrogens for puberty induction
- Participation in a clinical research study (currently or in the last 3 months) involving testosterone treatments or novel HRT formulations

5. Consent

Informed consent for each participant must be obtained prior to performing any trial related activities. Where a patient is currently receiving treatment with HRT, COC or testosterone and wishes to take part in the trial they will be asked to stop treatment for a 'washout' period of a minimum of 4 weeks prior to randomisation. Informed Consent must be taken prior to this washout period. Women will also be made aware that they may be randomised to receive the same treatment.

Consent for the trial will be taken in writing where possible. In light of the COVID-19 pandemic, alternative methods (e.g. electronic consent) may be used where a baseline face-to-face visit is not possible; local National Health Service (NHS) policy will dictate the method of consultation/visit and all consent taking will be in accordance with trial approvals, applicable regulatory policies and NHS guidelines.

All patients identified as being potentially eligible for the trial will be given a PIS and a member of the site research team will explain the trial to the patient. Where a face-to-face visit is not possible e.g. due to social distancing measures as a result of COVID-19, a copy of the PIS may be posted or provided electronically whichever is convenient to the patient. The potential participant will also be given contact details so that they can ask any questions they may have.

Consent may be obtained using a paper or an electronic system (e-consent). Where a face-to-face visit is not conducted and video or tele-consultations are conducted in accordance with local NHS Trust policy, e-consent will be used. The potential participant will be given the opportunity to ask questions throughout the process. Consent will be obtained by a member of the site research team in accordance with the delegation of responsibilities authorised by the Principal Investigator on the site delegation log. This will usually be by a medically qualified doctor, or where local trust policy allows this may be by a research nurse. **Eligibility for the trial must always be confirmed by a medically qualified doctor via the eligibility checklist.** Where a participant completes a 4-week washout period, eligibility will be re-confirmed prior to randomisation.

The consent documentation will also inform participants that their General Practitioner (GP) will be made aware of their participation in the trial.

5.1. Responsibilities

It is the responsibility of the Principal Investigator to ensure informed consent is obtained appropriately for all patients recruited at their site. A PIS will be provided to facilitate this process. Investigators or delegates will ensure that they adequately explain the aim, trial treatment, anticipated benefits and potential hazards of taking part in the trial to the participant. They will also stress that participation is voluntary and so the potential participant is free to decline participation and may withdraw from the trial at any time.

Patients who are identified in clinic as potentially being eligible for the trial will be asked by the investigator or delegate if they are interested in participating and given a copy of the PIS to take home to read. Those patients attending a clinic visit by telephone or video call, will be given the same information about the trial and if interested, sent a copy of the PIS by post. The PIS will also be available online. Patients will be given sufficient time to read the PIS and to discuss their participation with others (e.g. family members, GP or other healthcare professionals outside of the site research team, if they wish). Patients identified via other methods e.g. review of clinical notes, database searches (see Section 3.2), will be sent a copy of the PIS by post or email.

If the patient expresses an interest in participating in the trial, they will be asked to sign and date the latest version of the ICF.

The consent form will be completed in clinic or online. The participant must give explicit consent for the regulatory authorities, members of the research team and representatives of the Sponsor to be given direct access to the participant's medical records, and a copy of the signed ICF to be sent to the Nottingham Clinical Trials Unit (NCTU).

The Investigator or delegate will then sign and date the form (either physically or by countersigning online). A copy of the ICF will be given to the participant where completed face to face, or a copy can be downloaded by the participant if completed electronically. Copies of consent forms should be filed in the participants medical notes (where medical notes are electronic, a copy must be scanned (where paper) and uploaded into the patient's notes, or digitally uploaded (where electronic)), and the original kept in the Investigator Site File (ISF). Once the participant is enrolled onto the trial, the participant's unique trial identification number will be entered on the ICF maintained in the ISF. In addition, the participant will give their explicit consent for a copy of the signed ICF to be sent/made available to the NCTU for review.

Details of the informed consent discussions will be recorded in the participant's medical notes. This will include date of discussion, the name of the trial, summary of discussion, version number of the PIS given to participant and version number of ICF signed, and date consent received.

The consent form will include consent for the collection of contact details for the purpose of obtaining follow-up information and receiving trial communications. Where participants have given their consent to receive a summary of the trial results at the end of the trial their contact details will also be used for this purpose.

Throughout the trial the participant will have the opportunity to ask questions about the trial. Any new information that may be relevant to the participant's continued participation will be provided. Where new information becomes available which may affect the participants' decision to continue, participants will be given time to consider this and if they are happy to continue their consent will be re-confirmed. Reconfirmation of consent will be documented in the medical notes. The participant's right to withdraw from the trial will remain.

Electronic copies of the PIS and ICF will be available from the NCTU and via the trial website; it is the responsibility of the local site research team to ensure that all copies available locally are printed or copied onto the headed paper of the local institution.

Details of all participants approached about the trial will be recorded on the Participant Screening/Enrolment Log.

Patients previously on treatment who complete a washout period and are subsequently found to be ineligible for the trial after the washout period will not be randomised but their consent documentation will be retained and reasons for ineligibility will be recorded.

5.2. Optional consent for sub-study

Patients at selected sites will be asked to take part in an optional sub-study looking at bone metabolism and cardiovascular health. Patients will be asked to give consent to provide blood and/or urine samples for bone metabolism and cardiovascular biomarker analysis (Section 8.4). Consent for this study will be optional and patients will also be asked to consent to storage of some of their samples for future biomarker and genetic analysis (subject to further funding and ethical approval). Chromosomal analysis of women enrolled into the sub-study only, will be offered if not completed previously, and only for women with spontaneous POI.

5.3. GP Notification

Following randomisation and with the participant's prior consent, their GP will be informed that the participant is taking part in the trial. In accordance with usual clinical practice, prescriptions for further supplies of HRT or COC will ordinarily be issued by the participant's GP, therefore their GP will be informed of the treatment allocated at randomisation and they will be requested to issue further prescriptions in accordance with this allocation.

Participants randomised to HRT treatment will be required to pay a double prescription charge in some nations; in order to encourage adherence to randomised treatment allocation GPs will be encouraged to issue 6 monthly prescriptions for treatment to reduce frequency of prescription charges for participants in this group.

6. Enrolment and Randomisation

6.1. Enrolment

Once informed consent has been obtained, patients will be enrolled onto the trial. Patients currently receiving treatment with HRT, COC or testosterone will be asked to stop treatment for a 'washout' period of a minimum of 4 weeks prior to enrolment (upon completion of the washout eligibility will be re-confirmed).

After informed consent (and if required a washout period), baseline data will be collected by a member of the site research team and the participant will be enrolled using the online trial randomisation system. All participants are required to have had a DEXA bone density measurement of lumbar spine and hip in the 6 months prior to randomisation. Further information on baseline data collection requirements is detailed in Section 8.2.

An authorised member of the site research team will then log into the secure randomisation system and randomise the participant.

6.2. Randomisation

Eligible women will be individually randomised on a 1:1 ratio to one of the treatment groups using an online randomisation system developed and maintained by the NCTU. Access to the system will be granted by the NCTU in accordance with the roles delegated by the Principal Investigator on the Site Delegation Log.

Treatment will be assigned using a minimisation algorithm balancing on the following factors:

- Recruiting site
- Age at randomisation (≥18- <25 years or ≥25 - <40 years)
- Body mass index (<25 kg/m^2 or 25 - <30 kg/m^2 or ≥30 kg/m^2)
- Smoking status (quit smoking/never smoked or current smoker)
- Previous oestrogen treatment (Yes or No)

The minimisation algorithm will include a probabilistic element to allocation, making prediction of the allocated group virtually impossible.
For women randomised to HRT, participant preference will be taken into account when deciding which route of administration, oral or transdermal, to be used. Where there is no preference, the route will be randomly selected using an online randomisation system.

6.3. Blinding and concealment

Blinding of investigators and participants is not practical nor ethical due to the diversity in the range of treatment options, and the different contraceptive effect.

The blinding status of individuals involved in the management and delivery of the trial is detailed in Table 1.

Table 1: Blinding status of individuals involved in the trial

	Blinding status	Comments
Participant	Not blinded	Not practical / ethical due to the nature of the treatment. Participants will be informed which treatment they have been randomised to after randomisation.
Principal investigator and other site staff	Not blinded	Not practical /ethical due to the nature of the treatment.
Chief investigator(s)	Blinded	The Chief Investigators (CI) will remain blinded to treatment allocation overall (knowledge of treatment allocation is limited to participants at their own site). In instances where serious adverse events are reported, the CIs will become unblinded to complete the full causality assessment.
Database programmer	Not blinded	The database programmer will be responsible for the management of the randomisation system and will have access to unblinded datasets within the trial database.
POISE Trial Management staff within NCTU	Not blinded	POISE Trial Management staff within NCTU will have access to the unblinded datasets within the trial database including information on treatment adherence.
Data management	Not blinded	Data management staff will have access to the unblinded datasets within the trial database to ensure data quality and undertake central monitoring activities.
Trial statistician and Senior Trial Statistician	Blinded	The trial and senior trial statistician will not have access to treatment allocations or data which has the potential to unblind until after the first database lock for the analysis of the primary outcome at 2 years
Independent statistician	Not blinded	A statistician independent to the trial team will be responsible for the generation of closed reports for the Data Monitoring Committee (DMC) and other potentially unblinding data and will therefore be unblinded to trial treatments.

7. Trial treatment / intervention

7.1. Treatment

Participants will be randomised to either HRT or COC treatment.

Women already taking HRT or COC treatment are eligible to take part in the trial if they are willing to stop treatment for a washout period and potentially receive the same class of therapy again. The washout period must be for a minimum of 4 weeks, to allow time for their hormone levels to return to a pre-treatment level thus allowing a realistic assessment of symptoms and quality of life at baseline.

7.1.1. HRT

Women randomised to receive treatment with HRT should be prescribed oestradiol in a **daily dose** of 2mg orally, or a 50µg patch, or 1.5mg of gel, given continuously.

Women with a uterus will also be prescribed progestogen, taken cyclically or continuously. The formulation is not mandated. The preferred oral formulation is oestradiol 2mg with dydrogesterone 10mg (Femoston 2/10), and the preferred transdermal formulation is oestradiol 50µg with norethisterone 170µg patch (Evorel Sequi). Alternative formulations can be prescribed if required but must contain oestradiol as mandated above, for example oestradiol gel with micronised progesterone or LNG-IUS.

Women who are not using the LNG-IUS will be advised to use non-hormonal contraception if required.

7.1.2. COC

Women allocated the COC should be prescribed 30µg ethinyloestradiol with 150µg levonorgestrel (Microgynon 30 or equivalent) as an extended regimen; the suggested regimen is 63 days with 7 days hormone-free interval. Alternative formulations can be prescribed if required but must contain 30µg ethinyloestradiol in a monophasic formulation.

Use of a contraceptive patch or ring is not permitted.

Table 2: Summary of treatment options for initiating randomised treatment

Treatment Group	Permitted route(s) of administration	Formulation/Specified dose	Administration/additional information
COC	Oral	Monophasic tablets containing 30µg ethinyloestradiol	Tricycling for 63 days of tablets followed by a 4-7 day hormone-free interval, or flexible extended use, taking continuously with short breaks of 3-4 days if breakthrough bleeding occurs. The preferred regimen is tricycling for 63 days of tablets followed by a 7-day hormone-free interval.
HRT	Oral	Containing 17β-oestradiol. Starting dose: 2mg daily.	Women with POI and an intact uterus require progestogen to protect against endometrial hyperplasia; progestogen may be given cyclically or continuously.
	Transdermal	Containing 17β-oestradiol. Starting dose: Patch: 50µg oestradiol daily Gel: 1.5mg oestradiol daily	

7.1.3. Dosing schedule

HRT: Prescribed treatment to be taken continuously for up to 5 years, for a minimum duration of 2 years, if no contraindications arise.

COC: Prescribed treatment to be taken as an extended regimen for up to 5 years, for a minimum duration of 2- years, if no contraindications arise.

For both HRT and the COC, participants will be advised to remain on their allocated treatment for up to 5 years, for a minimum duration of 2 years.

7.1.4. Treatment if pregnant

Women who become pregnant will remain in the study but should stop taking their allocated treatment for the duration of their pregnancy. After birth, the site team should be notified of the date of delivery and the allocated hormonal treatment should be resumed following the advice of the participant's doctor. The site team will need to establish the date on which treatment re-commenced. Follow-up (DEXA bone measurements, clinical assessments and patient-facing questionnaires) should be resumed 1 year after delivery date. At each follow up timepoint participants will be asked their pregnancy status and, if applicable, pregnancy outcome.

7.2. Treatment Supply and Storage

7.2.1. Treatment Supplies

Following randomisation and treatment allocation, prescriptions for the randomised treatment will be initiated by the randomising clinician, with repeat prescriptions generated either from the participant's usual clinical care team which may be at the recruiting site or from the participant's GP, according to usual local practice for continuation of care.

Sites participating in this trial will be located in the UK. At the time of writing this protocol, participants living in Scotland and Wales are entitled to free prescriptions and COC and HRT will be prescribed in the usual manner.

In England, prescriptions for the COC are free whereas HRT prescriptions are typically charged, although an annual pre-payment certificate is available (£19.80 annually in 2023 prices) and should be recommended.

For any participant who is not eligible for free prescriptions nor pre-payment certificates, to try and minimise the costs for participants and encourage adherence to treatment allocation, clinicians will be encouraged, where possible to prescribe 6 months of treatment instead of the usual 3-month prescription (including repeat prescriptions).

The participant's GP will receive a letter informing them that their patient is participating in the study and will be requested not to change the regimen without consultation with the participant's consultant at the randomising hospital. This is to try and maximise treatment adherence and ensure that any dose or formulation changes are captured in the participant's health record at the randomising hospital.

7.2.2. Packaging and Labelling

In accordance with the Medicines and Healthcare products Regulatory Agency (MHRA) risk-adapted approach to the management of clinical trials of investigational medicinal products[38] this low risk Type A study does not require trial specific labelling. All HRT and COC treatments being used in this trial are

established treatments for oestrogen deficiency in young women and widely prescribed in primary and secondary care. HRT is being used within its licenced indication. For COC, this off-label use is established practice and supported by published guidelines[6].

7.2.3. Storage of Treatment

There are no trial-specific requirements for the storage of any of the treatments used in this trial. All treatment prescribed and dispensed for the purpose of the trial will originate from standard pharmacy stock which will be stored in accordance with the manufacturer's storage instructions as detailed in the applicable Summary of Product Characteristics (SmPC). Sites and/or local pharmacies will follow their own local policies for storage of medication.

7.3. Switching from randomised treatment allocation/changes to dosing

If a participant changes from the allocated treatment e.g. HRT to COC or changes the dose at any point during the study, this will be recorded in the electronic Case Report Form (CRF) but will not be considered a protocol deviation. Non-adherence with treatment allocation will be reviewed regularly by the Trial Management Group.

It is acknowledged that adherence with the randomised allocation will decrease over time, due to participant preference and life circumstances.

7.4. Treatment Interaction(s) or Contraindications

7.4.1. HRT

Patients receiving other drugs affecting BMD or estrogens for puberty induction are not eligible to take part in the trial (see section 4). Refer to the SmPC for the prescribed treatment and British National Formulary (BNF) for further details on interactions or contraindications.

7.4.2. COC

Patients receiving other drugs affecting BMD or estrogens for puberty induction are not eligible to take part in the trial (see section 4). Refer to the SmPC for the prescribed treatment and BNF and FSRH guideline[37] for further details on interactions or contraindications.

7.4.3. Testosterone

It is advised that participants in the POISE trial should not receive testosterone for at least the first 2 years following randomisation since this may be a confounding factor. However, treatment with testosterone remains at the decision of the treating clinician and the patient. If a participant does start testosterone during the trial, they will not be withdrawn and will continue to be followed-up. This will be recorded in the eCRF and the trial statistician will be made aware.

7.4.4. Changes in contraindications

If a participant develops a contraindication during the study resulting in a permanent or temporary change, or cessation of treatment, the participant should remain in the study for the purpose of follow-up data collection and any required changes to treatment documented in the patient's notes.

7.5. Accountability Procedures

There are no trial-specific accountability requirements; sites and/or local pharmacies will follow their own local procedures for recording treatments dispensed.

7.6. Treatment Modification

The prescribed formulations can be modified at the discretion of the randomising clinician following discussion with the participant. Any changes must remain within the constraints detailed above and not reduce the oestrogen dose.

If any dose modifications (increase or decrease) or formulation changes do occur these will be documented in the electronic Case Report Form (eCRF) and will not be considered as protocol deviations. The participant will remain in the study and will complete all remaining assessments and follow-up questionnaires as planned.

8. Trial procedures and assessments

8.1. Summary of assessments

A summary of the trial procedures and assessments is shown in Table 3. A detailed description of each assessment is provided in Section 8.2.
Follow-up assessments will follow the schedule shown in Table 3 until the final participant randomised has completed their 2-year follow-up assessment. This means that those participants recruited early in the trial will provide data annually up to 5 years.

Table 3: Summary of trial procedures and assessments

	Timepoint									
				Follow-up**						
	Pre-enrolment & Screening	**BL**	**0**	**3m**	**6m**	**1y**	**2y**	3y	4y	5y
ENROLMENT:										
Patient identification	X									
Informed consent	X									
Washout (if needed)	X									
Confirm eligibility		X								
Patient demographics		X								
Lifestyle factors e.g. smoking, alcohol, exercise habits		X		X	X	X	X	X	X	X
Use of medication e.g. prescribed, complementary and supplements		X		X	X	X	X	X	X	X
Randomisation			X							
TREATMENT / INTERVENTION:										
HRT			♦—	—	—	—	—	—	—	—♦
COC			♦—	—	—	—	—	—	—	—♦
ASSESSMENTS:										
BMD (DEXA scan) lumbar spine and hip		X				X	X			X
Quality of life (MENQOL-I)		X		X	X	X	X	X	X	X
Sexual function (SAQ)		X		X	X	X	X	X	X	X
Work productivity (WPAI)		X		X	X	X	X	X	X	X
Blood pressure and weight		X		X	X	X	X	X	X	X
Height		X								

Blood and urine samples for bone biomarkers*		X		X		X				
Blood sample for CVD biomarkers*		X		X		X	X	X	X	X
Karyotype analysis* (if clinically appropriate and if not done prior to entering the trial).		X								
Pregnancy status and outcome		X		X	X	X	X	X	X	X
Adverse effects of treatment				X	X	X	X	X	X	X
Change/cessation/satisfaction of treatment				X	X	X	X	X	X	X

*****only performed at selected sites
m = months, y = years, BL = baseline
** Duration of participation will be up to 5 years, for a minimum duration of 2 years

8.2. Schedule of Assessments

8.2.1. Enrolment and baseline

a) Identify potentially eligible patient

All potentially eligible patients should be approached and provided with information about the trial. This information will be entered onto the patient screening/enrolment log at each recruiting site. Patients that decline participation are not obliged to give a reason, however this will be recorded where this is known. Sites will be required to provide a summary of screening data on an ongoing basis during the recruitment period which will be reviewed regularly by the Trial Management Group (TMG) and oversight committees.

b) Informed Consent

Informed consent will be obtained as described in section 5. If a patient requires a washout period then eligibility will need to be re-confirmed prior to collecting baseline data.

c) Washout

Eligible women who are already on COC/HRT/testosterone treatment are required to stop treatment for a washout period of a minimum of 4 weeks. This is to ensure a return to pre-treatment hormone levels before performing baseline assessments.

d) Confirm Eligibility

Eligibility will be confirmed by the principal investigator or delegated trial doctor using the eligibility checklist form once informed consent and baseline data have been obtained.

e) Baseline data collection

After informed consent (and washout if applicable), baseline visit data will be collected by a member of the site research team and will include:

1. Patient demographics
2. Lifestyle factors e.g. smoking, alcohol, exercise habits
3. Use of medication e.g. prescribed, complementary and supplements
4. Pregnancy and breastfeeding
5. BMD (DEXA bone density measurement of lumbar spine and hip)
6. Quality of life (MENQOL-I questionnaire)
7. Sexual function (SAQ)
8. Work productivity (WPAI questionnaire)
9. Blood pressure (BP) and weight (refer to details below for BP measurement)
10. Height (measured at visit)
11. Blood and/or urine samples for bone and CVD biomarkers (selected sites only)

12. Karyotype analysis (selected sites only and if clinically appropriate and not done prior to entering trial.

Participants will have the option to complete each questionnaire electronically or by paper. Baseline questionnaires will be provided to participants by site staff either in a paper format or by providing access to an electronic format. Where paper questionnaires are used these will be entered into the eCRF by the site staff.

All BP measurements should be performed after the participant has been lying down at 45° for at least 5 minutes. Three measurements should then be taken, the first result discarded, and the 2nd and 3rd results used.

f) Randomisation

Randomisation of participants will occur as described in section 6.2

g) Prescribing of interventions / treatments

HRT will be prescribed in accordance with the manufacturer's instructions. For COC, off-label use is established practice and supported by published guidelines[6]. Sites and GP's will be advised to prescribe no less than 6 months of treatment (including repeat prescriptions) to help improve adherence to the allocated treatment.

8.2.2. Follow up

Follow up timepoints have been timed to coincide with routine clinic visits. Details of which procedures/assessments are required at each timepoint is provided in Table 3. Questionnaires, and blood and/or urine samples for bone and CVD biomarkers (if applicable) are for the purpose of the study whereas all other assessments are considered standard care.

Where possible, all follow-up BP measurements should be performed as part of a clinic visit using the instructions detailed in Section 8.2.1. Where clinical outcomes data collection at clinic visits is not possible, collection from local GP practices and pharmacies is permitted. In this case a letter could be sent, by the site team, to the GP requesting that the surgery contact the participant and undertake the BP procedure. Alternatively, the patient themselves could contact the surgery or pharmacy to undertake the BP procedure. A collection form will be provided to the participant for the GP practice or pharmacy to record the data. The participant will need to send this form back to the study team or NCTU. This will be monitored centrally. GP practices and pharmacies will also be asked to record participant's weight and height on the collection form.

Participants can complete their follow up questionnaires at a clinic appointment, or remotely. If not completed in clinic, questionnaires will be provided to participants by post or email. Where paper questionnaires are used these will be entered into the eCRF by the site staff. If required, participants will receive messages to remind them to complete the questionnaires.

Participants will be required to give their consent for collection of contact details for the purpose of issuing follow-up questionnaires and important trial communications (e.g., reminder messages) as part of the eligibility assessment and informed consent process.

Data on participants including diagnosis of cancer, CVD, cognitive impairment, bone fractures and mortality will be collected at 5 years from NHS data sources (NHS England at the time of writing this protocol), using validated diagnostic codes and hospital episode data.

8.3. Trial Procedures

The availability of DEXA scan appointments at some sites may result in time delays, specifically if the participant requires a baseline scan. If the participant has not had a DEXA scan in the 6 months prior to the date of randomisation then baseline assessments and randomisation should be delayed until this can be performed. Where possible all DEXA scans throughout the duration of the study for a participant should be completed on the same machine.

Throughout the study, reminders and updates will also be shared with participants via a range of media such as newsletters, websites, text message reminders and social media platforms.

8.4. Sub-study

Participants recruited at selected sites will be invited to consent to collection of blood and/or urine samples, which will contribute to the assessment of the relative impact of the COC and HRT on early changes in bone turnover and the cardiovascular effects of hormone treatment.

Bone markers: Blood samples for the analysis of liver function tests, bone profile, and 1,25 Vitamin D and blood and/or urine samples for the analysis of bone metabolism markers will be collected at baseline, 3 months and 12 months.

Blood samples for the analysis of liver function tests, bone profile and 1,25 Vitamin D will be analysed following the same process as routine clinical samples using local laboratories. Results will be entered into the eCRF by site staff.

Blood and/or urine samples for the analysis of selected bone metabolism markers will be collected and processed as detailed in the site sample processing manual. Processed samples will be stored and then analysed by a central laboratory. Results will be transferred directly to a member of the NCTU once available and not reported until the end of the study.

Cardiovascular markers: Blood samples for the analysis of fasting lipids, fasting glucose, HBA1c, insulin like growth factor, and renal function will be collected at baseline, 3 months and 12 months and then annually (up to 5 years, until the final main trial participant has reached the 2 year timepoint). These samples will be analysed following the same process as routine clinical samples using local laboratories. Results will be entered into the eCRF by site staff.

A Karyotype analysis will also be offered at baseline, as part of routine care, in women with spontaneous POI, if this has not been carried out prior to entering the trial.

Consent for long-term storage of blood and blood serum samples collected at the same timepoints (baseline, 3 months, 12 months and annually up to 5 years) will also be sought from the same sub-set of participants to allow further analysis, subject to additional funding and ethical approval. Samples will be kept for up to 5 years post trial completion date. Specific consent will be obtained to undertake additional genetic analysis subject to additional funding. Samples will be collected and processed as detailed in the site sample processing manual. Processed blood and blood serum samples will be stored centrally. Any analysis will be undertaken by a central laboratory. Results will be transferred directly to a member of the NCTU once available.

8.5. Treatment discontinuation

Participants who discontinue the study treatment or change treatment for any reason will continue to be followed up in accordance with the trial schedule and continue to provide trial data, including

completion of follow-up questionnaires for use in the analysis, unless they are unwilling to do so. All data collected will be used, and any participants that discontinue study treatment will be reminded of the importance of continuing to complete study questionnaires/assessments.

Reasons for trial treatment discontinuation may include participant decision or significant adverse events.

8.6. Withdrawal from study

Participants are free to withdraw from the trial at any time and for any reason. Reasons for withdrawal from the trial may include participant decision or death. Participants who stop taking study treatment will continue to complete follow-up assessments and will not be considered to have withdrawn from the trial.

8.6.1. Withdrawal prior to randomisation

Any patients that request to withdraw their consent **prior to randomisation** will be withdrawn completely from the trial; they will not be randomised, and follow-up questionnaires will not be issued.

8.6.2. Discontinuation and withdrawal post randomisation

Participants may withdraw their consent for follow-up and/or other trial related activities/receiving trial communications. The NCTU must be informed of all requests by participants to stop their involvement in the trial; appropriate action will be taken to ensure that the participant's wishes are followed.

Sites will be trained to determine which activities participants may wish to withdraw from.

Withdrawal type	Withdrawal procedure	Use of data
Discontinue follow-up questionnaires	Any participant that requests to discontinue from trial questionnaires will be marked as withdrawn from questionnaire collection on the trial database and no further contact will be made with the participant for the purpose of obtaining questionnaire follow-up data.	Any data collected prior to participant withdrawal will be retained and used.
Discontinue from collection of blood and/or urine samples for bone / CVD biomarkers	Any participant that requests to no longer take part in the sub-study to provide blood and/or urine samples will be marked as withdrawn from sample collection on the trial database.	Any samples taken prior to withdrawal will be retained and used.
Discontinue trial related communication e.g. newsletters, study summaries.	Any participant that requests to discontinue receiving trial communications e.g. newsletter, results summaries, will be marked as	Any data collected prior to participant withdrawal will be retained and used.

	withdrawn from trial related communication on the trial database.	
Collection of data from medical records and/or NHS England	Any participant that requests to discontinue collection of routine data will be directed to the national data opt out service.	Any data collected prior to participant withdrawal will be retained and used.
Full trial withdrawal	Any participant that requests to have no further involvement in the trial will be marked as withdrawn on the trial database.	Any data collected prior to participant withdrawal will be retained and used.

If site staff are made aware of a participant's withdrawal of consent for any trial activities, the PI or delegate should record this in the CRF as soon as possible (and within 24 hours) to ensure the correct procedures are followed by NCTU and the site team. Participants will be asked their reason(s) for withdrawal but are not obliged to provide these.

Withdrawn participants will not be replaced. Data collected prior to withdrawal will be retained and used in the analysis. It should be made clear to any participant withdrawing consent for further data collection that data pertaining to safety will continue to be collected for regulatory purposes and will be included in any safety analysis and the final analysis. In addition, if any significant new information becomes available with regard to the treatment they received in the trial, it may be necessary to contact them in the future.

8.7. Losses to follow-up

If contact cannot be made and the participant has not withdrawn their consent to participation in the trial, outcome data will be obtained from medical records and/or NHS England where possible. If it is not possible to obtain the primary outcome the participant will be designated as lost to follow-up for the primary outcome.

9. Adverse Event Reporting

9.1. Definitions

Adverse Event (AE)	Any untoward medical occurrence in a clinical trial participant administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including abnormal laboratory findings), symptom or disease temporally associated with the use of an investigational medicinal product, whether or not related to the investigational medicinal product.
Adverse Reaction (AR)	All untoward and unintended responses to an IMP related to any dose administered. An AE judged by either the reporting Investigator or Sponsor as having causal relationship to the IMP qualifies as an AR. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

Serious Adverse Event (SAE)	Any untoward medical occurrence or effect that: • Results in death • Is life-threatening* • Requires hospitalisation or prolongation of existing hospitalisation • Results in persistent or significant disability or incapacity • Is a congenital anomaly/birth defect • Or is otherwise considered medically significant by the Investigator** The term severe is often used to describe the intensity (severity) of a specific event. This is not the same as serious, which is based on participants/event outcome or action criteria. * Life threatening in the definition of an SAE refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe. ** Medical judgment should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life threatening or do not result in death or hospitalisation but may jeopardise the participant or may require intervention to prevent one of the other outcomes listed in the definition above, should be considered serious.
Serious Adverse Reaction (SAR)	An Adverse Reaction which also meets the definition of a Serious Adverse Event
Unexpected Adverse Reaction (UAR)	An AR, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator Brochure for an unapproved IMP or Summary of Product Characteristics (SmPC) for a licensed product). When the outcome of an AR is not consistent with the applicable product information the AR should be considered unexpected.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A SAR that is unexpected i.e. the nature, or severity of the event is not consistent with the applicable product information. A SUSAR should meet the definition of an AR, UAR and SAR.

9.2. Adverse Events and reporting requirements/procedures

The collection and reporting of Adverse Events (AEs) will be in accordance with the Medicines for Human Use Clinical Trials Regulations 2004 (and subsequent amendments).

All IMPs (HRT and COC) being used in this trial are licensed and have well characterised safety profiles.

All preparations of HRT used in this trial are licensed for use for oestrogen deficiency symptoms in post- and peri-menopausal women.

Whilst the COC is licensed as an oral contraceptive it is not licensed specifically for treating oestrogen deficiency and POI. It is however widely, prescribed for the treatment of POI; this off-label use is standard practice and all treatments used in the trial have well-established safety profiles.

In order to provide secondary outcome data about the adverse events associated with the use of the COC or HRT, only specified adverse events will be collected.

The following expected AEs will be participant-reported and collected using a questionnaire at 3, 6, 12 months and yearly up to 5 years, depending on the point at which the participant joins the trial. If a

clinic visit is not possible or a participant fails to attend, questionnaires will be provided to participants electronically so they can be completed remotely.

Participants will be asked at each follow-up visit if they have experienced any of the following:

- Unscheduled bleeding
- Breast pain
- Nausea
- Headaches
- Skin or hair changes (acne/facial spots, greasy or oily skin, hair loss/hair thinning, hirsutism (=excessive hair growth on face or body))
- Dysmenorrhoea (period pains)
- Menorrhagia (heavy periods)
- Premenstrual syndrome (PMS)
- VTE or pulmonary embolism (PE)
- Broken bone(s)

Where any of the above events result in an admission to hospital or prolongation of a hospital stay this will also require reporting on a SAE form by the Principal Investigator or delegate (see section 9.3).

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) as per NCTU standard practice.

9.3. Serious Adverse Events and reporting requirements

Investigators will report all AEs that they become aware of that meet the definition of an SAE, including those adverse events collected via patient questionnaire as mentioned in section 9.2.

AEs defined as serious, and which require reporting as an SAE should be reported on an SAE form. When completing the form, the Investigator will be asked to define the causality and severity of the AE. The SAE form must be sent to the NCTU as soon as possible and no later than 24 hours after first becoming aware of the event (or obtaining the follow-up information from the participant).

On receipt NCTU will allocate each SAE a unique reference number which will be forwarded to the site as proof of receipt within 1 working day. If confirmation is not received within 1 working day sites should contact the NCTU. The SAE reference number will be quoted on all correspondence and follow-up reports regarding the SAE and filed with the actual SAE in the Site File and TMF.

Sites

For SAE Forms completed by someone other than the Investigator, the Investigator will be required to countersign the original SAE Form to confirm agreement with the causality and severity assessments. The form should then be returned to NCTU and a copy kept in the Site File. Investigators should also report SAEs to their own Trust in accordance with local practice.

On becoming aware that a participant has experienced an SAE, the Investigator (or delegate) must complete, date and sign a SAE form. The form should be e-mailed to NCTU using the following e-mail address:

FOR SAE REPORTING ONLY: nctu-sae@nottingham.ac.uk

NCTU

On receipt of an SAE Form seriousness and causality will be reviewed independently by the medical monitor responsible for determining causality assessments. An SAE judged by the Investigator or medical monitor (in this case the CI), to have a reasonable causal relationship with the trial medication will be regarded as a Serious Adverse Reaction (SAR). The medical monitor will also assess all SARs for expectedness. If the event meets the definition of a SAR that is unexpected (i.e. is not defined in the Reference Safety Information (RSI) –see Section 9.4) it will be classified as a SUSAR.

9.3.1. Provision of follow-up information

For all serious adverse events identified and reported during the study, participants should be followed up until resolution or stabilisation of the event. Follow-up information should be provided on a SAE Follow-up form.

9.4. Reference Safety Information

The Reference Safety Information (RSI) will be the SmPC's as described below:

For the COC treatment participants prescribed Microgynon 30 or an equivalent the RSI will be Microgynon 30 tablets (levonorgestrel, ethinylestradiol) Bayer plc.

For the HRT treatment participants prescribed an oral formulation the RSI will be Femoston 2/10mg film-coated tablets (oestradiol, dydrogesterone) Mylan Products Ltd.

For the HRT treatment participants prescribed a transdermal patch the RSI will be EVOREL SEQUI (estradiol hemihydrate, norethisterone acetate) Theramex UK Limited.

For the HRT treatment participants prescribed a transdermal gel the RSI will be Oestrogel Pump-Pack 750 micrograms/actuation gel (estradiol) Besins Healthcare (UK) Limited.

Any updates to the RSI will be reviewed annually in line with the Development Safety Update Report (DSUR).

9.5. Reporting period

Details of AEs (except those listed is section 9.2) will be documented and reported from the date of commencement of protocol defined treatment until 2 years after randomisation of the last participant.

9.6. Monitoring pregnancies for potential Serious Adverse Events

There is no requirement to monitor pregnancies for potential Serious Adverse Events.

9.7. Reporting to the Competent Authority and Research Ethics Committee

9.7.1. Suspected Unexpected Serious Adverse Reactions

On becoming aware of a SUSAR, the Trial Manager (or delegate) will notify the Sponsor as soon as possible. The Sponsor will report a minimal data set of all individual events categorised as a fatal or life threatening SUSAR to the MHRA and Research Ethics Committee (REC) within 7 days. Detailed follow-up information will be provided within an additional 8 days. All other events categorised as SUSARs will be reported within 15 days.

SUSARs will be unblinded to allow reporting to REC and the MHRA.

9.7.2. Serious Adverse Reactions

The NCTU will report details of all SAEs and SARs (including SUSARs) to the MHRA and REC annually from the date of the Clinical Trial Authorisation, in the form of a Development Safety Update Report (DSUR).

9.7.3. Adverse Events

Details of all AEs will be reported to the MHRA on request.

9.7.4. Other safety issues identified during the course of the trial

If any urgent safety measures are taken the CI shall immediately and, in any event, no later than 3 days from the date the measures are taken, give written notice to the MHRA, the relevant REC and the sponsor, of the measures taken and the circumstances giving rise to those measures

9.8. Investigators

Details of all SUSARs and any other safety issue which arises during the course of the trial will be reported to Principal Investigators. A copy of any such correspondence should be filed in the Investigator Site File.

9.9. Data Monitoring Committee

The independent DMC will review all SAEs.

9.10. Reporting to third parties

No reporting of adverse events to third parties is expected. Any safety issues identified during the course of the trial will be notified to the MHRA.

10. Data Handling and Record Keeping

10.1. Source Data

Source data is defined as all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial.

In order to allow for the accurate reconstruction of the trial and clinical management of the participant, source data will be accessible and maintained.

Source data is kept as part of the participant's medical notes generated and maintained at site. Each site will record the location of source data at their site using a source data location log prior to commencing recruitment. Data that are not routinely collected elsewhere may be entered directly onto the eCRF; in such instances the eCRF will act as source data and this will be clearly defined in the source data location log and recorded. Some recruiting centres may initially record trial information into a source data worksheet; where this has been used this will be noted.

For this trial, source data refers to, though is not limited to, the participants' medical notes, laboratory blood results, data recorded directly into the CRF, source data worksheets and questionnaires. (Questionnaires completed by participants on paper will be considered source data, if questionnaires are completed online by the participant the data in the eCRF will be considered source data).

Where paper follow-up questionnaires are issued to participants these will be entered into the eCRF by a member of the research team at site for data entry and will be considered source data. Where

follow-up is obtained via telephone, this data will be entered directly into the eCRF or collected on paper proforma (where direct eCRF entry is not possible) by a member of the NCTU and will be considered source data.

10.2. Routine data

At 5 years follow-up, key outcomes e.g. cancer, bone fractures and cardiovascular events will be collected from NHS England routine data (or equivalents in devolved nations) using validated diagnostic codes and hospital episode data.

10.3. Electronic Case Report Form (eCRF) Completion

Data will be reported using an eCRF. Reported data will be consistent with the source data and any discrepancies will be explained. Staff delegated to complete the eCRF will be trained to adhere to ICH-GCP guidelines and trial specific guidance on the completion of the eCRF.

For instances where data may not be directly entered onto the eCRF, a set of trial specific data collection worksheets will be provided to the sites to facilitate collection of trial data prior to the site entering the data onto the eCRF.

In all cases it remains the responsibility of the site's Principal Investigator to ensure that the eCRF has been completed correctly and that the data are accurate as evidenced by the signature of the site's Principal Investigator on the eCRF.

10.4. Data Management

Details about data handling will be specified in the Data Management Plan (DMP). This will include the agreed validation specification which will validate data for consistency and integrity as it is entered.

All trial data will be entered onto a trial specific database through the eCRF with participants identified only by their unique trial number and initials. The database will be developed and maintained by NCTU. Access to the database will be restricted and secure. Any missing or ambiguous data will be queried with the site via the eCRF, sites should respond to the data queries in a timely manner, ideally within 2 weeks of the query being raised. All access and data transactions will be logged in a full audit trail.

Participant's eCRF data will be reviewed and frozen on an ongoing basis once they are deemed to have a complete set of data that has passed data validation checks (i.e. there are no data queries outstanding). Once all participant data have been frozen and the statistical analysis plan has been finalised, the trial database will be locked (set to read only). This will be done prior to the data analysis. It is planned that there will be 2 database locks after 2 and 5 years follow up.

10.5. Archiving

It is the responsibility of the Principal Investigator to ensure all essential trial documentation and source documents (e.g. signed ICFs, ISFs, copies of CRFs etc.) at their site are securely retained for at least 25 years after the end of the trial. Documents will be archived following regulatory requirements and any local procedures. No documents will be destroyed without prior approval from the Sponsor.

10.6. Data sharing

Individual participant medical information obtained as a result of this trial is considered confidential and disclosure to third parties is prohibited with the exceptions noted in this protocol.

Participants' contact details, including name, address, telephone/mobile number and email will be shared between participating sites and NCTU for the purposes of issuing questionnaires and electronic reminders (text/email) for the trial.

Minimal linked anonymised data (participation identification code, initials and date of birth), used for labelling of laboratory samples (bone metabolism markers), will also be shared with the analysing laboratory.

Any personal data will be held in a secure database using encryption, with restricted password protected access. Only appropriate members of the participating site team and NCTU research team will have access to these data.

Participant confidentiality will be further ensured by utilising identification code numbers to correspond to treatment data in computer files.

Data generated as a result of this trial will be available for inspection on request by University College London, NCTU, the REC, local R&D departments and the regulatory authorities.

Anonymised participant data may be shared with researchers external to the trial research team in accordance with the NCTU's data sharing procedure. All requests for data should be sent to the NCTU.

11. Quality control and quality assurance

11.1. Site Set-up and Initiation

All participating Principal Investigators will be asked to sign the necessary agreements and supply a current signed CV to the NCTU. All members of the site research team will also be required to sign a **site delegation log and training log**. Prior to commencing recruitment all sites will undergo a process of initiation and will have completed Good Clinical Practice (GCP) training (PIs will be required to evidence this with GCP certification). Key members of the site research team will be required to attend either a meeting or a teleconference covering aspects of the trial design, protocol procedures, Adverse Event reporting, collection and reporting of data and record keeping. Sites will be provided with an ISF containing essential documentation, instructions, and other documentation required for the conduct of the trial. The NCTU must be informed immediately of any change in the site research team.

11.2. Monitoring

Monitoring will be carried out as required following a risk assessment and as documented in the trial monitoring plan. The NCTU will be in regular contact with the site research team to check on progress and address any queries that they may have. The trial team will check incoming eCRF data for adherence with the protocol, data consistency, missing data and timing. Sites will be asked for missing data or clarification of inconsistencies or discrepancies. Additional monitoring (including on-site visits) may be triggered, for example by poor CRF return, poor data quality, lower/higher than expected SAE reporting rates, excessive number of participant withdrawals or deviations. If an on-site monitoring visit is required, NCTU will contact the site to arrange a date for the proposed visit and will provide the site with written confirmation. Investigators will allow NCTU trial/monitoring staff access to source documents as requested.

Sites will be requested to provide copies of signed ICFs and other documentation for in-house review for central monitoring for all participants. This will be detailed in the monitoring plan.

11.3. Audit and Inspection

The Principal Investigator will permit trial-related monitoring, quality checks, audits, ethical reviews, and regulatory inspection(s) at their site, providing direct access to source data/documents. The Principal Investigator will comply with these visits and any required follow up. Sites are also requested to notify NCTU of any MHRA inspections.

The Trial Master File and evidence of audits will be made available upon request for regulatory inspections.

11.4. Notification of Serious Breaches

In accordance with Regulation 29A of the Medicines for Human Use (Clinical Trials) Regulations 2004 (and its amendments) the Sponsor of the trial is responsible for notifying the licensing authority in writing of any serious breach of the conditions and principles of GCP in connection with that trial or the protocol relating to that trial, within 7 days of becoming aware of that breach.

For the purposes of this regulation, a "serious breach" is a breach which is likely to affect to a significant degree the safety or physical or mental integrity of the subjects of the trial; or the scientific value of the trial. Sites are therefore requested to notify NCTU of any suspected trial-related serious breach of GCP and/or the trial protocol. Where NCTU is investigating whether or not a serious breach has occurred, sites are also requested to cooperate with NCTU in providing sufficient information to report the breach to the MHRA where required and in undertaking any corrective and/or preventive action.

Sites may be suspended from further recruitment in the event of serious and persistent non-adherence with the protocol and/or GCP, and/or poor recruitment. Any major problems identified during monitoring may be reported to the trial oversight committees (TMG, TSC, DMC), the REC and the relevant regulatory bodies. This includes reporting serious breaches of GCP and/or the trial protocol to the REC and MHRA.

12. End of Trial Definition

The end of trial will be **the date of final database lock**. NCTU will notify the MHRA and REC that the trial has ended within 90 days of the end of trial.

Where the trial has terminated early, NCTU will inform the MHRA and REC within 15 days of the end of trial. NCTU will provide them with a summary of the clinical trial report within 12 months of the end of trial.

13. Statistical Considerations

13.1.1. Power Calculations / sample size calculation

The original sample size was powered to detect a mean difference in lumbar spine BMD (g/cm^2) of 0.05 between the HRT and the COC group at 2 years post-randomisation to ensure that the trial preserves at least the effect observed in a similar previous study[17]. This target difference also represented an absolute change thought to be the least significant change in spine BMD that is unlikely to be due to the precision error of the test. The precision error is best described using the smallest detectable difference, rather than the coefficient of variation, and for lumbar spine a change of BMD of at least +/-0.05g/cm^2 is considered clinically significant[39,40]. Assuming that the lumbar spine BMD in each group varies with a standard deviation (SD) of 0.15, observed from 112 comparable patients in an unpublished cohort of POI women and the previous trial[17], a total of 382 patients (191 per group)

were required to detect the between–group difference of 0.05 g/cm^2 (a small effect size of 0.333) with a 90% power, 5% 2-sided significance level and 1:1 allocation. Attrition had been a major problem in studies of similar population, so we allowed for up to 20% loss to follow-up, requiring 480 patients to be recruited.

This sample size was expected to still have >80% power to detect a medium effect size (between-group mean difference of 0.075 g/cm^2, SD=0.15) between each HRT route (oral or transdermal) and the COC, assuming the proportion in one route is between 20% to 50%.

The above sample size was calculated without accounting for correlation between repeated outcome measures. At the design stage, it was decided not to account for the repeated measures design in the sample size calculation to be conservative as it was assumed that the efficiency gained by repeated measures would be offset by possible efficiency loss attributable to misspecification of any of the sample size parameters due to uncertainty about the assumptions.

At a review after 18 months of recruitment it was recommended by the Trial Steering Committee (TSC) that the sample size should be readjusted to account for the correlation between repeated outcome measures. No outcome data was available to the TSC or investigators when the decision was made. The revised sample size uses the same parameters as the original sample size calculation but are based on a mixed-effect model analysis of repeated measures data including a baseline and two repeated post-randomisation outcome measurements, with contrasts to assess treatment effects at each time point. The calculation assumes a compound symmetry covariance structure (exchangeable), with a common variance (σ^2) for the outcome measure and a common correlation (ρ) between repeated follow-up assessments (autocorrelation). For each value of σ^2, the required sample size increases with the correlation ρ between follow-up measures such that a correlation of 1 gives a sample size equivalent to two-sample t-test. A value of ρ=0.4 to 0.6 is typical in many studies. At the time of the review, there was insufficient accrued data to calculate the autocorrelation, so a conservative autocorrelation between follow-up measures of 0.6 was chosen. The sample size required for analysis to estimate the same effect as the original calculation, with 90% power, is 304. Allowing for 20% loss to follow-up requires the recruitment of 380 participants. To achieve 80% power and allowing for 20% loss of primary outcome data, 286 participants would be required.

13.2. Definition of Outcome Measures

13.2.1. Primary outcome measures

The primary outcome is absolute BMD (g/cm^2) at 2 years from the date of randomisation assessed by a standard DEXA scan of the lumbar spine.

13.2.2. Secondary outcome measures

The secondary outcomes will be measured at different stages throughout the trial. Further information can be found in Section 2.2.2.

13.3. Analysis of Outcome Measures

The analysis and reporting of the trial will be in accordance with CONSORT guidelines. A full statistical analysis plan (SAP) will be developed and agreed prior to database lock for the analysis of the primary outcome at 2 years.

Appropriate descriptive statistics (mean, standard deviation, median, lower and upper quartiles, minimum, maximum or frequencies and percentages) for the demographic and clinical outcome measures at baseline will be used to assess balance between the randomised arms at baseline, but no formal statistical comparisons will be performed. Baseline characteristics will also be descriptively compared between those randomised and those analysed to see if the attrition has introduced any imbalances. Descriptive statistics appropriate for the outcome will also be presented for all outcomes at all collected time points by treatment group.

The primary comparative analysis will be based on intention-to-treat, analysing participants in the groups to which they were randomised. The evaluation of the primary outcome of BMD in the lumbar spine at 2 years will be performed using a mixed effects model to allow for all available follow-up data (1 year, 2 year) to be used, adjusted for the baseline BMD in the lumbar spine and the minimisation variables as fixed effects except site which will be entered as a random effect. The model will include a treatment by time interaction to obtain the estimates of treatment effect at each follow-up time, with 2 years being the primary evaluation. An interaction between each covariate and time will be added to get a different adjustment for each covariate at each time point. The estimated between group effect will be presented using the difference in means, with a 95% confidence interval. The primary treatment comparison will be the contrast between allocated treatment groups at 2 years.

Sensitivity analyses for the primary outcome will include:

- Complete case analysis based on observed outcome data at 2 years
- Use of multiple imputation with auxiliary variables (if applicable) not included in the primary analysis also included in the imputation model.
- Adjustment for any other baseline variable (if applicable) with marked imbalance between the two treatment groups

Supplementary analyses for the primary outcome will investigate potential effects of compliance with allocated treatment using complier average causal effect (CACE) analysis (or per-protocol analysis should there be high crossover rates making CACE identification impossible) and estimate an overall time-averaged between-group effect over the 2 years to compare the overall rate of change in BMD between groups.

Main analyses of secondary outcomes will be based on intention-to-treat, analysing participants in the groups to which they were randomised, regardless of adherence with allocation. Sensitivity analyses (as described for the primary outcome) may also be performed for key secondary outcomes such as blood pressure, MENQOL-I, and BMD at 1 year and 2 years. Ordered logistic regression models will be used to compare the total lumbar spine and hip T-score categories (≤-2.5, > -2.5 to ≤ -1, > -1) between groups, adjusted for the minimisation variables; however, should there be only a small number of participants in one of the categories then categories will be collapsed into two for analysis and logistic regression used. Between-group comparison of secondary outcomes will be based on an appropriate regression for the outcome adjusted for the minimisation variables and baseline outcome measure for continuous variables if available. For repeated measures, appropriate mixed-effect models for the outcome (linear for continuous outcomes and logistic for binary), adjusted for the minimisation variables, will be used to estimate the difference between groups at each time point. Supplementary analysis of key secondary outcomes may also be conducted according to actual treatment used if the percentage of women who stop using their allocated treatment or switch to the other treatment is high.

Adverse events will be presented descriptively only and will be summarised according to both the allocated group and the treatment received.

Mixed-effect models, with similar adjustments as described for analysis of the primary outcome, will be used to compare BMD and key secondary outcomes between each route of HRT administration (oral and transdermal) HRT and COC and between oral HRT and transdermal HRT (non-randomised comparison). Key secondary outcomes to be compared will be specified in the SAP.

13.3.1. Planned Randomisation Methodology

Randomisation of participants will occur as described in section 6.2.

13.3.2. Planned Interim Analysis

There is no planned interim analysis of treatment effectiveness. However, an internal pilot phase was built into the trial to allow a feasibility assessment which will examine recruitment, retention and adherence. The original stop-go criteria are shown in Table 4, assessed 12 months after the first site opening. A review was held at this point, involving the TSC, DMC and funder. Recruitment was in the Red category, whilst Adherence and Retention were in the Amber category. A recovery plan was agreed, including a revised sample size (detailed in section 13.1). A further feasibility review is scheduled for month 36 of recruitment, using the same stop=go criteria. . Recruitment will be measured against the overall recruitment target at month 36. Retention will be determined by return of the MENQOL-I questionnaire at 6 months and adherence will be measured by use of allocated treatment at 6 months.

Table 4: Stop-Go Criteria for the internal pilot

	Black (stop study)	Red (proceed with recovery plan)	Amber (proceed with changes)	Green (proceed)
Trial recruitment	<50%	50-79%	80-99%	≥100%
Recruitment rate relative to overall target at month 36 of recruitment (participants per month per site)	<0.35	≥0.35-0.55	0.56-0.69	≥0.70
Adherence (on allocated treatment at 6 months)	<50%	50-79%	80-99%	100%
Retention (return of MENQOL-I questionnaire at 6 months)	<50%	50-79%	80-99%	100%

The second feasibility review will be undertaken by the funder, TSC and DMC and if recruitment, retention or adherence meet the red or amber categories then additional strategies will be put in place to attempt to improve these. The review is planned to be conducted without knowledge of treatment allocation. However, the DMC will be able to access data by treatment allocation if requested.

13.3.3. Planned Final Analyses

Analysis of the primary outcome and secondary outcomes up to 2 years will be performed after all data up to the 2 year point has been collected and cleaned and the SAP finalised.

Analysis of the secondary outcomes between 3 and 5 years will be performed after all data up to the 5 year point has been collected and cleaned.

13.3.4. Planned Subgroup Analyses

Subgroup analyses for the primary outcome will be performed according to age at randomisation (≥18-<25 years or ≥25 - <40 years)), BMI (<25 kg/m^2 or 25 - <30 kg/m^2 or ≥30 kg/m^2), hysterectomy (yes or no), smoking status (quit smoking/never smoked or current smoker), and previous oestrogen

treatment (Yes or No), by including appropriate interaction terms in the mixed effect model. The trial is not powered to detect any interactions hence the subgroup analyses will be treated as exploratory.

14. Trial Organisational Structure

14.1. Sponsor

The trial is sponsored by University College London (UCL).

14.2. Trials Unit

The trial is co-ordinated by the NCTU based at the University of Nottingham.

14.3. Trial Management Group

The TMG will be responsible for the day-to day management of the trial. Membership includes (but is not limited to) the CIs, Trial Statistician, Trial Manager and Data Manager. Other relevant members of the trial team will be invited to TMG meetings as required. The TMG will ensure high quality trial conduct, to time and within budget, monitor all aspects of the conduct and progress of the trial, ensure that the protocol is adhered to and take appropriate action to safeguard participants and the quality of trial itself. The TMG will also be responsible for ensuring project milestones are achieved. The TMG will meet regularly throughout the duration of the trial.

14.4. Trial Steering Committee

A TSC will be established and will include an independent chair, independent and non-independent members, and a patient representative. The CI will also be a member of the TSC. The role of the TSC is to maintain oversight of the trial, monitor progress and provide advice to the research team.

The TSC will provide independent oversight of the trial and will meet at least annually or more often as required, either face-to-face or by tele- or videoconference. The TSC will consider and act, as appropriate, upon the recommendations of the DMC, and in accordance with the TSC Charter, and ultimately carries the responsibility for deciding whether the trial needs to be stopped on grounds of safety or efficacy.

14.5. Data Monitoring Committee

Reports will be supplied in confidence to an independent DMC, which will be asked to give advice on whether the accumulated data from the trial, together with the results from other relevant research, justifies the continuing recruitment of further participants. The DMC will operate in accordance with a trial specific charter based upon the template created by the Damocles Group. The DMC will meet initially during the trial set-up period to agree the protocol and content of the DMC charter and then annually unless there is a specific reason for additional meetings. After completion of the 2 year follow up, the meeting frequency will be reviewed by the DMC and if agreed reduced further.

Additional meetings may be called if recruitment is much faster than anticipated and the DMC may, at their discretion, request to meet more frequently or continue to meet following completion of recruitment. An emergency meeting may also be convened if a safety issue is identified. The DMC will report directly to the chair of the TSC who will convey the findings of the DMC to the TSC, TMG, funders and Sponsor as applicable.

14.6. Finance

This trial is funded by the National Institute for Health and Care Research (NIHR) Health Technology Assessment (HTA) programme (funding award number: NIHR128757).

14.7. Participants payment and stipends

Participants will not be paid to participate in the trial. However, participants will receive a voucher when clinical outcomes have been collected at 3 months, 6 months, 1 year and 2 year time-point. The vouchers will equate to approximately £110 per participant (£20 at 3 and 6 months, £35 at 1 and 2 years). . This is a recompense towards the additional costs associated with missing work etc to attend trial-related appointments.

15. Ethical Considerations

The trial will be performed in accordance with the recommendations guiding physicians in biomedical research involving human participants, adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, June 1964, and its amendments (website: http://www.wma.net/en/30publications/10policies/b3/index.html).

The trial will be conducted in accordance with the UK Policy Framework for Health and Social Care Research 2018, the applicable UK Statutory Instruments, (which include the Medicines for Human Use Clinical Trials 2004 and subsequent amendments and the Data Protection Act 2018), Human Tissue Act 2004 and Human Tissue (Scotland) Act 2006 (if applicable) and Guidelines for Good Clinical Practice (GCP). This trial will be carried out under a Clinical Trial Authorisation in accordance with the Medicines for Human Use Clinical Trials regulations. The protocol will be submitted to and approved by the REC prior to circulation.

16. Confidentiality and Data Protection

Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the Data Protection Act 2018.

Participant names and contact details will be collected for the purpose of contacting participants to obtain follow-up information and to send important trial communications (e.g. reminder messages for trial questionnaires) in accordance with the schedule outlined in this protocol.

To enable outcome data to be collected from NHS England, primary (NHS number or equivalent) and secondary (date of birth, and full postcode) linkage variables will be collected from participants. Any routine data requested will only contain the minimum required information for the purposes of the trial and for accurate data linkage. Any patient identifiable data received (e.g. NHS number) will be deleted once the patient data has been linked between the data sets and received from the applicable database. Patient identifiable information will not be used in the datasets analysis.

Participants will always be identified using only their unique trial identification number and initials on the CRF/eCRF and in correspondence between NCTU and the participating site.

Blood samples taken for the purpose of the trial which are not processed as routine clinical samples (selected sites only) will be labelled as trial samples and identified by their unique trial identification number, initials and date of birth. Results will be transferred from the analysing laboratory to a member of the NCTU team. Further details on sample processing will be provided in the site sample processing manual.

Participants will give their explicit consent for a copy of any paper versions of the informed consent form to be sent to the NCTU or uploaded into a secure area of the trial database. Finger, stylus or mouse drawn signatures used on e-consent forms will be stored within a secure area of the trial

database. Receipt of consent forms will be used to perform in-house monitoring of the consent process.

The Investigators must maintain documents not for submission to NCTU (e.g. Participant Identification Logs) in strict confidence. In the case of specific issues and/or queries from the regulatory authorities, it will be necessary to have access to the complete trial records, provided that participant confidentiality is protected.

NCTU will maintain the confidentiality of all participant's data and will not disclose information by which participants may be identified to any third party other than those directly involved in the treatment of the participant and organisations for which the participant has given explicit consent for data transfer (e.g. laboratory staff, competent authority, Sponsor). Representatives of NCTU and Sponsor may be required to have access to participant's notes for quality assurance purposes, but participants should be reassured that their confidentiality will be respected at all times.

17. Insurance and Indemnity

University College London will act as sponsor for the trial. Delegated responsibilities will be assigned to the NHS Trusts taking part and NCTU.

University College London hold insurance against claims from participants for injury caused by their participation in the clinical trial. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, as this clinical trial is being carried out in a hospital, the hospital continues to have a duty of care to the participant of the clinical trial. University College London does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or otherwise.

Participants may also be able to claim compensation for injury caused by participation in this clinical trial without the need to prove negligence on the part of University College London or another party. Participants who sustain injury and wish to make a claim for compensation should be advised to do so in writing in the first instance to the Chief Investigator, who will pass the claim to the Sponsor's Insurers, via the Sponsor's office. Hospitals selected to participate in this clinical trial shall provide clinical negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary shall be provided to University College London, upon request.

The University of Nottingham has appropriate and typical insurance coverage in place (including, but not limited to Clinical Trials, Professional Indemnity, Employer's Liability, and Public Liability policies) in relation to the Institution's Legal Liabilities arising from the University's activities and those of its staff, whilst conducting University business and research activity.

University College London (the sponsor) is independent of any pharmaceutical company, and as such it is not covered by the Association of the British Pharmaceutical Industry (ABPI) guidelines for participant compensation.

18. Publication Policy

The dissemination of the proposed research findings will be via a published HTA monograph, research papers for publication in peer reviewed journals, presentation at scientific conferences and communication of the findings to groups involved in guideline development. Manuscripts will be

prepared by the CI, deputy CI and TMG and authorship will be determined by mutual agreement. The TSC and DMC will be given opportunity to comment on the manuscripts prior to submission.

Any secondary publications and presentations prepared by Investigators must be reviewed by the CI, deputy CI and NCTU. Manuscripts must be submitted to either party in a timely fashion and in advance of being submitted for publication, to allow time for review and resolution of any outstanding issues. Authors must acknowledge that the trial was performed with the support of University College London.

All publications will acknowledge the support of the NIHR in funding this trial.

Trial participants will be asked whether or not they would like to receive a summary of the research findings, following the publication of the results. The research summaries will also be provided to the PPI partners, who can circulate to their membership.

Publications arising from further research conducted using shared trial datasets should appropriately acknowledge the trial investigators and funder.

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