PROTOCOL TITLE The effect of low-dose rhythmic 17-β-estradiol administration on bone turnover in postmenopausal women

Protocol ID	**NL83336.018.23**
Short title	**Rhythmic 17-β-estradiol and bone health**
Acronym	**REBEL-study**
EudraCT number	**2022-003866-19**
Version	**2.0**
Date	**09-03-2023**
Coordinating investigator	**E.M. Speksnijder** **PhD candidate** **K2-283** **020-5666071** **e.m.speksnijder@amsterdamumc.nl**
Principal investigator(s) (in Dutch: hoofdonderzoeker/ uitvoerder)	**Prof. dr. P.H.L.T. Bisschop** **Internist-endocrinologist** **K2-283** **020-5666071** **p.h.bisschop@amsterdamumc.nl**
Sponsor (in Dutch: verrichter/opdrachtgever)	**Amsterdam UMC, location AMC** **Meibergdreef 9, 1105 AZ Amsterdam**
Subsidising party	**Amsterdam UMC, location AMC** **Meibergdreef 9, 1105 AZ Amsterdam**
Independent expert (s)	**Dr. M.R. Soeters** **Internist-endocrinologist** **K2-283** **020-5666071** **m.r.soeters@amsterdamumc.nl**
Laboratory sites	**Central Diagnostic Laboratory, Amsterdam UMC, location AMC. Meibergdreef 9, 1105 AZ**

	Amsterdam
Pharmacy	**Pharmacy Amsterdam UMC, location AMC** **Meibergdreef 9, 1105 AZ Amsterdam**

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Head of Department: **Prof. dr. J.M Prins** **Internist-infectiologist**		
Principal Investigator: **Prof. dr. P.H.L.T. Bisschop** **Internist-endocrinologist**		

TABLE OF CONTENTS

1. INTRODUCTION AND RATIONALE 10
2. OBJECTIVES 12
3. STUDY POPULATION 14
3.1 Population (base) 14
3.2 Inclusion criteria 14
3.3 Exclusion criteria 14
3.4 Sample size calculation 15
4. TREATMENT OF SUBJECTS 16
4.1 Investigational product/treatment 16
4.2 Use of co-intervention (if applicable) 16
4.3 Escape medication 17
5. INVESTIGATIONAL PRODUCT 18
5.1 Name and description of investigational product(s) 18
5.2 Summary of findings from non-clinical studies 18
5.3 Summary of findings from clinical studies 18
5.4 Summary of known and potential risks and benefits 19
5.5 Description and justification of route of administration and dosage 19
5.6 Dosages, dosage modifications and method of administration 19
5.7 Preparation and labelling of Investigational Medicinal Product 20
5.8 Drug accountability 20
6. NON-INVESTIGATIONAL PRODUCT 21
6.1 Name and description of non-investigational product(s) 21
6.2 Summary of findings from non-clinical studies 21
6.3 Summary of findings from clinical studies 21
6.4 Summary of known and potential risks and benefits 21
6.5 Description and justification of route of administration and dosage 21
6.6 Dosages, dosage modifications and method of administration 21
6.7 Preparation and labelling of Non Investigational Medicinal Product 22
6.8 Drug accountability 22
7. METHODS 23
7.1 Study parameters/endpoints 23
7.1.1 Main study parameter/endpoint 23
7.1.2 Secondary study parameters/endpoints (if applicable) 23
7.1.3 Other study parameters (if applicable) 24
7.2 Randomisation, blinding and treatment allocation 24
7.3 Study procedures 24
7.4 Withdrawal of individual subjects 26
7.4.1 Specific criteria for withdrawal (if applicable) 26
7.5 Replacement of individual subjects after withdrawal 26
7.6 Follow-up of subjects withdrawn from treatment 26
7.7 Premature termination of the study 26

8 SAFETY REPORTING 27
8 Temporary halt for reasons of subject safety 27
8.1 AEs, SAEs and SUSARs 27
8.1.1 Adverse events (AEs) 27
8.1.2 Serious adverse events (SAEs) 27
8.1.3 Suspected unexpected serious adverse reactions (SUSARs) 28
8.2 Annual safety report 29
8.3 Follow-up of adverse events 29
8.4 Data Safety Monitoring Board (DSMB) 30
9 STATISTICAL ANALYSIS 31
9.1 Primary study parameter(s) 31
9.2 Secondary study parameter(s) 32
9.3 Other study parameters 32
9.4 Interim analysis (if applicable) 32
10 ETHICAL CONSIDERATIONS 33
10.1 Regulation statement 33
10.2 Recruitment and consent 33
10.3 Objection by minors or incapacitated subjects (if applicable) 33
10.4 Benefits and risks assessment, group relatedness 33
10.5 Compensation for injury 34
10.6 Incentives (if applicable) 35
11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION 36
11.1 Handling and storage of data and documents 36
11.2 Monitoring and Quality Assurance 36
11.3 Amendments 36
11.4 Annual progress report 37
11.5 Temporary halt and (prematurely) end of study report 37
11.6 Public disclosure and publication policy 37
12. STRUCTURED RISK ANALYSIS 39
12.1 Potential issues of concern 39
12.2 Synthesis 39
13. REFERENCES 41

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)**
AE	**Adverse Event**
AR	**Adverse Reaction**
AxMP	**Auxiliary Medical Product**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)**
(S)AE	**(Serious) Adverse Event**
SmPC	**Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
UAVG	**Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG**
WMO	**Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: Postmenopausal estrogen deficiency leads to accelerated bone resorption, resulting in an increased risk of osteoporosis and osteoporotic fractures (1). Continuous estrogen replacement therapy (ERT) with 17-β-estradiol is an effective treatment for the prevention of osteoporosis in postmenopausal women (2). ERT normalizes bone turnover by attenuating bone remodelling, reflected by a decrease of bone turnover markers procollagen type 1 N propeptide (P1NP) and C-terminal telopeptide of type 1 collagen (CTX)(3). Notably, estrogen replacement can also induce an anabolic effect in bone tissue, which is not well studied. The anabolic effect of 17-β-estradiol occurs when continuous estrogen replacement therapy is first initiated. After initiation of 17-β-estradiol therapy, serum concentrations of bone formation marker P1NP rise, while a decrease of bone resorption marker CTX is observed (4, 5),suggesting that an increase of serum 17-β-estradiol generates an initial anabolic response in bone tissue. Similarly, when serum 17-β-estradiol levels physiologically increase during the luteal phase of the menstrual cycle (Fig. 1), a simultaneous increase of P1NP is observed, in combination with a decrease of CTX levels (5). Therefore, we hypothesize that, by mimicking the rhythmic estrogen concentrations during the menstrual cycle in postmenopausal women, a subsequent rhythmic rise of bone formation marker P1NP can be induced, and as a result, improved bone formation.

In addition to the regulation of bone metabolism, estrogens regulate key features of glucose metabolism and energy expenditure (4). Estrogen deficiency after menopause leads to accumulation of visceral and hepatic fat, as well as altered insulin sensitivity and insulin secretion (5). A previous meta-analysis demonstrated that estrogen replacement therapy (ERT) has beneficial effects on insulin resistance and fasting glucose levels in postmenopausal women. We hypothesize that rhythmic transdermal estrogen administration may produce larger beneficial effects on glucose regulation and on liver steatosis (CAP score) than continuous transdermal estrogen administration, in postmenopausal women.

The increase in visceral fat that is observed in estrogen deficiency is partly the result of classical (genomic) estrogen effects on adipose tissue. Estrogen therapy in postmenopausal women decreases the expression of genes involved in lipogenesis (including LPL and FAS) (4). However, it is unknown how physiological 17-β-estradiol will affect this gene expression on a transcriptional level. We will perform ChipSeq and RNA Seq analysis of the effect of rhythmic 17-β-estradiol versus low-dose and standard-dose continuous 17-β-estradiol on transcriptional regulation in subcutaneous adipose tissue.

Objective: 1. To investigate the effect of low-dose rhythmic transdermal estrogen replacement, versus low-dose and standard-dose continuous transdermal estrogen

replacement on changes of serum P1NP and CTX over time.
2. To assess the effect of low-dose rhythmic transdermal 17-β-estradiol on fasting glucose, fasting insulin, insulin resistance (HOMA-IR), post-OGTT glucose and insulin and liver steatosis (CAP score), versus continuous low-dose and standard-dose continuous transdermal 17-β-estradiol.
3. To assess the effect of low-dose rhythmic transdermal 17-β-estradiol versus continuous low-dose and standard-dose continuous transdermal 17-β-estradiol on sleep quality.
4. In a subgroup: To assess the effect of low-dose rhythmic transdermal 17-β-estradiol versus continuous low-dose and standard-dose continuous transdermal 17-β-estradiol on 17-β-estradiol transcriptional regulation in adipose tissue.

Study design: Randomized controlled intervention trial (open-label)

Study population: Healthy postmenopausal women (final menstrual cycle more than 1 year and less than 10 years prior to inclusion, FSH>30 IU/L) aged 45-60 years, without a contra-indication for therapy with estrogens and/or progestogens.

Intervention: Participants will receive one of the following treatments for a duration of 16 weeks:

- Rhythmic 17-β-estradiol: Alternating 4-week cycles consisting of transdermal 17-β-estradiol 25µg/24hrs for two weeks, followed by two weeks of transdermal 17-β-estradiol 50µg/24hrs. 17-β-estradiol therapy will be combined with continuous oral micronized progesterone 100mg once daily.
- Low-dose continuous 17-β-estradiol: Continuous transdermal 17-β-estradiol 25µg/24hrs, combined with continuous oral micronized progesterone 100mg daily once daily.
- Standard-dose continuous 17-β-estradiol: Continuous transdermal 17-β-estradiol 50µg/24hrs, combined with continuous oral micronized progesterone 100mg daily once daily.

Main study parameters/endpoints: Mean change of serum P1NP concentrations from baseline until 16 weeks of treatment.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

Extent of burden:

For screening purposes, blood samples will be collected at baseline (3 tubes) and participants' medical history and family history will be collected. The participants' height, weight and blood pressure will be measured.

After inclusion, subjects will receive 17-β-estradiol treatment. Blood will be drawn every other week from baseline until week 16 to measure P1NP and CTX (1 tube at each time point).

Additionally, a fasting plasma glucose, estradiol, insulin, and an oral glucose tolerance test (OGTT) will be performed at baseline and after 16 weeks of treatment (4 tubes), and a fibroscan will be performed (10-minute ultrasound).
A chronotype questionnaire, sleep quality questionnaire and menopausal symptoms questionnaire will be filled in at baseline and after 16 weeks of treatment.

Optionally, participants will undergo two subcutaneous fat biopsies: One fat biopsy at baseline and one after 16 weeks.

Benefits: Participation in the trial does not involve an expected individual benefit for the participant other than relieving possible climacteric symptoms. However, there might be a group benefit if rhythmic low-dose estrogen therapy leads to increased bone formation in postmenopausal women.

Risks: Blood draws may cause bruising at the site of the punctured vein. Participants may experience side effects of the transdermal 17-β-estradiol treatment. The most common side effects are rash at the site of the transdermal patch (20.8%), redness at the site of the patch (8.5%), itchiness at the site of the patch (19.8%), headache (7.8%), and breast tenderness (6.6%) (SmPC systen). Progesterone may cause the following side effects: vaginal bleeding, breast tenderness, nausea, dizziness, somnolence (1-4 hours after administration), headache (SmPC uterogestan). There is no excess risk of venous thromboembolism in transdermal 17-β-estradiol users (6). Hormone replacement therapy for less than 1 year does not lead to increased risk of breast cancer (7)(SmPC Systen).

Risks fat biopsy: Participants will experience discomfort during the application of local anaesthesia, but thereafter the procedure is painless. A subcutaneous hematoma will develop after the fat biopsy, but this will gradually resolve. The subcutaneous fat biopsy can possibly cause bleeding or an infection around the site of the biopsy.

1. INTRODUCTION AND RATIONALE

Estrogens play a major role in the regulation of bone metabolism. Postmenopausal estrogen deficiency leads to accelerated bone turnover, resulting in an increased risk of osteoporosis and osteoporotic fractures (1). Continuous estrogen replacement therapy (ERT) with 17-β-estradiol is an effective treatment for the prevention of osteoporosis in postmenopausal women (2), which has a known antiresorptive effect, characterized by a decrease of the bone resorption marker C-terminal telopeptide type 1 collagen (CTX), as well as the bone formation marker procollagen type 1 N propeptide (P1NP) (3).

Notably, estrogen replacement can also induce an anabolic effect in bone tissue, which is not well studied. The anabolic effect of 17-β-estradiol occurs when continuous estrogen replacement therapy is first initiated. After initiation of 17-β-estradiol therapy, serum concentrations of bone formation marker P1NP rise, while a decrease of bone resorption marker CTX is observed (8, 9), suggesting that an increase of serum 17-β-estradiol generates an initial anabolic response in bone tissue. This observation is also seen during the menstrual cycle of premenopausal women. When 17-β-estradiol levels physiologically increase during the luteal phase of the menstrual cycle (Fig. 1), an anabolic response is observed, characterized by an increase of P1NP and a decrease of CTX levels (9). Taken together, we hypothesize that, by mimicking the rhythmic estrogen concentrations during the menstrual cycle, a subsequent rhythmic anabolic effect can be induced, and as a result, improved bone formation in postmenopausal women.

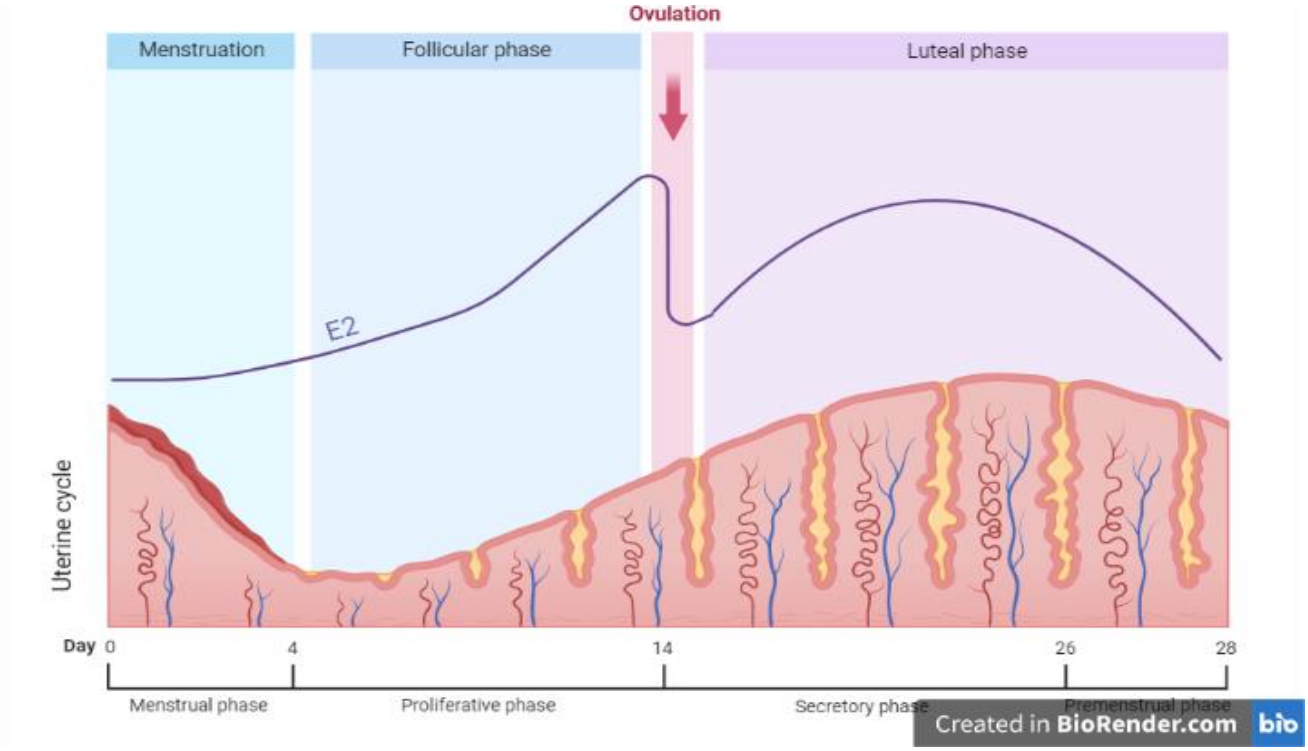
In support of our hypothesis, a previous clinical trial in women with premature ovarian failure has shown that alternating dose transdermal estrogen replacement therapy led to increased P1NP concentrations, decreased CTX concentrations and higher bone mineral density compared to standard estrogen replacement therapy (10). However, in this trial, a high dose of estrogens was used, which is unsuitable for postmenopausal women according to the guidelines for postmenopausal hormone replacement. In the present study, we will investigate the effect of low-dose, rhythmic transdermal estrogen replacement on changes of bone turnover markers P1NP and CTX over time. As the anabolic effects of 17-β-estradiol on bone tissue are not yet fully understood, we aim to further improve understanding of these physiological anabolic effects of estrogen on bone turnover in postmenopausal women.

In addition to the regulation of bone metabolism, estrogens regulate key features of glucose metabolism and energy expenditure (4). Estrogen deficiency after menopause leads to accumulation of visceral and hepatic fat, decreased energy expenditure and decreased lipolysis, as well as altered insulin sensitivity and altered insulin secretion (5). A previous meta-analysis (11) demonstrated that postmenopausal hormone replacement therapy (HRT) has beneficial effects on insulin resistance and fasting glucose levels in postmenopausal

women. Oral HRT produced larger beneficial effects on insulin resistance and fasting glucose than transdermal HRT. However, transdermal estrogen was administered continuously in all studies, and not in a physiological rhythmic manner (11). We hypothesize that rhythmic transdermal estrogen administration may produce larger beneficial effects on glucose regulation than continuous transdermal estrogen administration in postmenopausal women. In the present study, we will therefore additionally study the effect of rhythmic transdermal estrogen replacement on blood glucose and blood insulin in the fasting state, after an oral glucose tolerance test (OGTT) and on liver steatosis (CAP score).

The increase in visceral fat that is observed in estrogen deficiency is partly the result of classical (genomic) estrogen effects on adipose tissue. Estrogen therapy in postmenopausal women decreases the expression of genes involved in lipogenesis (including LPL and FAS) (4). However, it is unknown how physiological 17-β-estradiol will affect this gene expression on a transcriptional level. We will perform ChipSeq and RNA Seq analysis of the effect of rhythmic 17-β-estradiol versus low-dose and standard-dose continuous 17-β-estradiol on transcriptional regulation in subcutaneous adipose tissue.

Figure 1. 17-β-estradiol (E2) across the normal menstrual cycle



2. OBJECTIVES

Primary Objective:
To investigate the effect of low-dose rhythmic transdermal 17-β-estradiol on serum P1NP (marker of bone formation), versus continuous low-dose and standard-dose continuous transdermal 17-β-estradiol administration. The endpoint is the interaction between treatment and time on serum P1NP levels. (Does the change over time differ between the treatment arms?)

Secondary Objective(s):
To investigate the effect of low-dose rhythmic transdermal 17-β-estradiol on serum CTX (marker of bone resorption), versus continuous low-dose and standard-dose continuous transdermal 17-β-estradiol administration. The endpoint is the interaction between treatment and time on serum CTX levels. (Does the change over time differ between the treatment arms?)

To assess the effect of low-dose rhythmic transdermal 17-β-estradiol on fasting glucose, fasting insulin, insulin resistance (HOMA-IR), and post-OGTT glucose and insulin, versus continuous low-dose and standard-dose continuous transdermal 17-β-estradiol. The endpoint is change in fasting glucose, fasting insulin, insulin resistance (HOMA-IR), and 2 hours post-OGTT glucose and insulin in relation to baseline after 16 weeks of treatment.

To assess the effect of low-dose rhythmic transdermal 17-β-estradiol on liver steatosis (CAP score) in relation to baseline after 16 weeks of treatment.

Exploratory Objective(s):
To assess the effect of low-dose rhythmic transdermal 17-β-estradiol versus continuous low-dose and standard-dose continuous transdermal 17-β-estradiol on sleep quality. The endpoint is sleep quality (PSQI), chronotype (MCTQ) and menopausal symptoms (Greene Climacteric Scale) after 16 weeks in relation to baseline.

In a subgroup: To assess the effect of low-dose rhythmic transdermal 17-β-estradiol versus continuous low-dose and standard-dose continuous transdermal 17-β-estradiol on 17-β-estradiol transcriptional regulation in adipose tissue using ChipSeq and RNA seq analysis (hypothesis-generating).

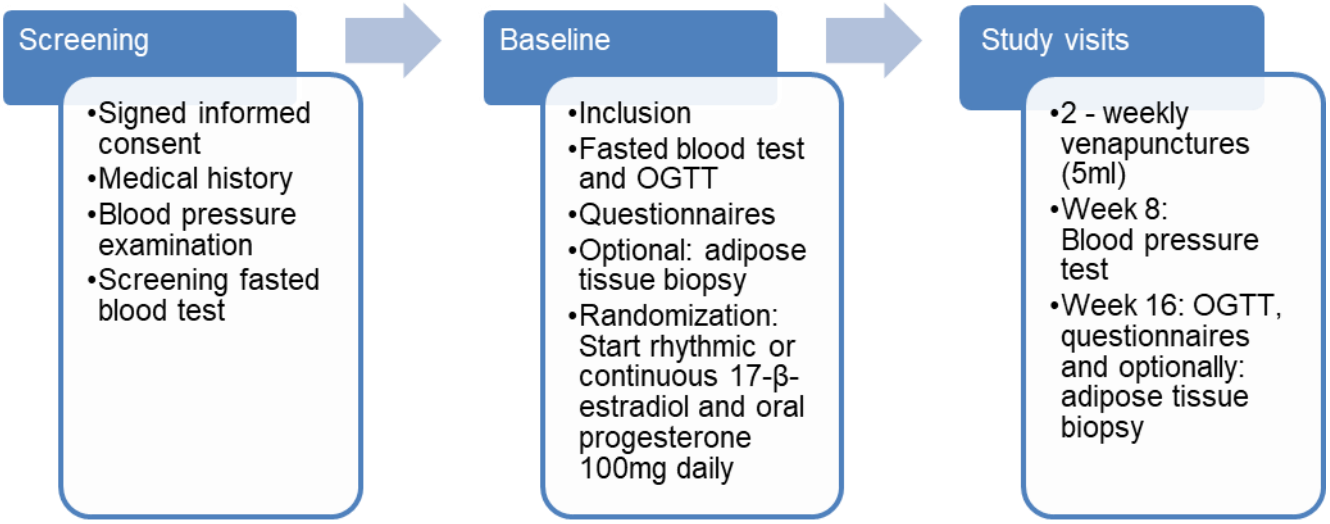
STUDY DESIGN

We will conduct a monocenter open-label randomized controlled trial, investigating the effect of rhythmic 17-β-estradiol (alternating 25 and 50 ug/24hr in four 4-week cycles) versus low-dose (25 ug/24hr) and standard-dose (50 ug/24hr) continuous 17-β-estradiol on bone turnover markers and secondary outcome parameters. The standard-dose continuous 17-β-estradiol (50ug/24hr) is a standard registered treatment for treatment of peri-menopausal complaints and prevention of osteoporosis.

All subjects will additionally be treated with progesterone 100mg daily for endometrial protection. The study will be set at Amsterdam UMC, location AMC and the study will have a duration of 16 weeks after inclusion.

An open-label parallel design was chosen, because the primary and secondary endpoints of this trial are objective outcomes measured in the laboratory of endocrinology. The analyst performing the laboratory assessments will be blinded to the treatment allocation.

Flowchart of study procedures:



3. STUDY POPULATION

3.1 Population (base)

We will include 48 healthy postmenopausal female subjects (16 in each group), aged 45-60 years.

3.2 Inclusion criteria

To be eligible to participate in this study, a subject must meet all the following criteria:

- Postmenopausal, defined as final menstrual cycle more than 1 years prior to inclusion and FSH>30 IU/L
- Final menstrual cycle < 10 years prior to inclusion
- Age 45-60 years

3.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

• Contra-indication for estrogen and/or progesterone therapy: Presence or suspicion or history of breast cancer, endometrial cancer, ovarian cancer, presence or history of venous thromboembolism, arterial thrombosis (e.g. myocardial infarction, angina pectoris) inherited or acquired thrombophilia, presence of liver disease, untreated endometrial hyperplasia, abnormal vaginal bleeding, porphyria, uncontrolled or severe hypertension)
• First-grade family member with inherited thrombophilia or history of VTE under the age of 60 years
• Hysterectomy
• Premature menopause (menopause age <40 years)
• Known hypersensitivity to the excipients in the estradiol patch: acrylate copolymer, polyethylene terephthalate, α-tocopherol, soy allergy or peanut allergy (component of progesterone capsule)
• Hormonal contraception or hormone replacement therapy use (estradiol with or without progesterone) in the past 12 months
• Presence or history of any clinically relevant metabolic, endocrinological, hepatic, renal, cardiovascular, gastrointestinal, or respiratory conditions, history of bone disease or bone marrow disease, known vitamin D deficiency (25-OH vitamin D <30 nmol/L)
• Recent fracture (<12 months)
• BMI <20 or BMI ≥30

- Use of drugs including herbal medicine known to affect bone metabolism (e.g. corticosteroids) or to interfere with cytochrome P450 enzyme (CYP) pathways. Exceptions are occasional use of paracetamol, ibuprofen, acetylsalicylic acid or topical medication
- For adipose tissue biopsy: anticoagulant treatment, allergy to lidocaine

3.4 Sample size calculation

The sample size calculation is based on the primary outcome measure P1NP (marker for bone formation) as we expect to see differences in serum P1NP between groups, in combination with minimal differences of serum CTX between groups.

A sample size of 16 subjects per group is needed to detect a difference in means of serum P1NP levels with a 0.05 significance level and 80% power between 3 groups. The sample size was calculated using nQuery 8.5.1.
Sample size calculation: When the sample size is 16, a repeated measures analysis of variance (repeated measures ANOVA) with a 0.05 significance level will have 80% power to detect a difference in mean P1NP across the 9 levels of the repeated measures, with a variance of means of 16.6, a standard deviation of 33, and a between level correlation of 0.87.

The variance of means and standard deviation are based on previous literature (8, 9). The between-level correlation of 0.87 is based on raw data from a previous study from our study group, investigating the effect of 2 weeks of 50ug/24hr 17-β-estradiol treatment on P1NP levels in postmenopausal women (9).

4. TREATMENT OF SUBJECTS

4.1 Investigational product/treatment

The investigational medical product is transdermal (17-β-)estradiol.

Group 1: The rhythmic 17-β-estradiol group will receive a transdermal patch for two weeks that releases 25ug/24hrs of 17-β-estradiol, and a patch of 50ug/24hrs for 2 weeks in each 4-week cycle.
This treatment reflects a more physiological estrogen concentration compared to continuous estrogen administration, because it reflects the alternating 17-β-estradiol concentrations during the menstrual cycle.

Group 2: The continuous low-dose 17-β-estradiol group will receive a transdermal patch that releases 25ug/24 hrs of 17-β-estradiol administered continuously during the 16 weeks of treatment. A dose of 25ug/24 hrs is used off-label in the treatment of menopausal symptoms.

Group 3: The continuous standard-dose 17-β-estradiol group will receive the standard therapy for prevention of osteoporosis. A transdermal patch that releases 50ug/24 hrs of 17-β-estradiol will be administered continuously during the 16 weeks of treatment.

4.2 Use of co-intervention (if applicable)

Participants are allowed to continue their usual medication regime during the trial. All participants will receive progesterone 100mg daily for endometrial protection.

Participants starting a new medical therapy during the trial are permitted to continue participating in the trail, as long as these medications do not the criteria of prohibited medications in this section. Starting a prohibited medical therapy during the trial will lead to discontinuation of study treatment.

<u>Prohibited medications:</u>

• Use of drugs including any herbal medicine known to interfere with cytochrome P450 enzyme (CYP) pathways. Exceptions are occasional use of paracetamol, ibuprofen, acetylsalicylic acid or topical medication.
• Use of drugs that affect bone metabolism such as steroids

• Use of drugs (other than the IMP and auxiliary medical product) that affect glucose metabolism

Participants aged ≥50 will be advised to use vitamin D supplements 800IE a day according to the Health Council of The Netherlands (12). Participants will be instructed not to make changes to their diet and the frequency and intensity of physical activity during the study.

4.3 Escape medication

Occasional use of paracetamol, ibuprofen, acetylsalicylic acid or topical medication is permitted.

5. INVESTIGATIONAL PRODUCT

5.1 Name and description of investigational product(s)

Name IMP: (Systen) estradiol patches 50 µg/24hr, patches for transdermal use.
Active substance: 17-β-estradiol
Formulation: Transdermal patch

The medication is used within authorized indication for prevention of osteoporosis. However, the administration timing will be altered in the rhythmic 17-β-estradiol group, to reflect a more physiological estrogen concentration as seen during the menstrual cycle.

5.2 Summary of findings from non-clinical studies

Not relevant, the IMP is an authorized medical product and is used for the prevention of osteoporosis.

5.3 Summary of findings from clinical studies

Standard-dose 17-β-estradiol: See SmPC “Systen” for information about standard-dose 17-β-estradiol therapy (page 1-2, 13-14).

Low-dose 17-β-estradiol: The low-dose 17-β-estradiol will be administrated using half a matrix patch (25ug/24hr). Participants will cut the patch in half to obtain administer a dose of 25µg/24hrs. This half-patch dose is used in the Netherlands for treatment of menopausal symptoms. Cutting the patch in half will lead to administration of half the dose of the full patch, this is used off-label in the Netherlands for treatment of menopausal symptoms (13). The 17-β-estradiol drug amount is uniformly distributed over the 17-β-estradiol matrix patch (14).

A low-dose patch of estradiol 25ug/24hr is available for osteoporosis prevention in other European countries, for example, in Belgium (Dermestril 25ug/24hr, see SmPC “Dermestril”). In the United States of America, 17-β-estradiol is registered for osteoporosis prevention for dosages of 25ug/24hr and 14ug/24hr (see SmPC “Menostar”).

Rhythmic dose 17-β-estradiol: Rhythmic doses of transdermal 17-β-estradiol have been used before in clinical trials to assess the effect on bone metabolism in two 12-month clinical trials (10, 15). Furthermore, some hormonal contraceptives are registered in rhythmic-dose oral estrogen preparations (e.g. Qliara, Trigynon)(Farmacotherapeutisch Kompas).

5.4 Summary of known and potential risks and benefits

See SmPC “Systen”, page 8-12

5.5 Description and justification of route of administration and dosage

The rhythmic 17-β-estradiol group will receive a transdermal patch for two weeks that releases 25ug/24hrs of 17-β-estradiol, and a patch for two weeks that releases 50ug/24hrs in each 4-week cycle. The patch needs to be replaced twice a week. The first patch is replaced after 4 days and the second patch is replaced after 3 days.

This treatment reflects physiological fluctuations of the estrogen concentration as seen during the menstrual cycle.

5.6 Dosages, dosage modifications and method of administration

Group 1: The rhythmic 17-β-estradiol group will receive a transdermal patch for two weeks that releases 25ug/24hrs of 17-β-estradiol, and a patch of 50ug/24hrs for 2 weeks in each 4-week cycle. The patch needs to be replaced twice a week. The first patch is replaced after 4 days, and the second patch is replaced after 3 days. Participants will cut the patch in half to obtain administer a dose of 25µg/24hrs*.

Group 2: The continuous 17-β-estradiol 25ug/25hr group will receive a transdermal patch that releases 25ug/24 hrs of 17-β-estradiol will be administered continuously during the 16 weeks of treatment. Participants will cut the patch in half to obtain administer a dose of 25µg/24hrs*. The patch needs to be replaced twice a week (first patch is replaced after 4 days, second patch is replaced after 3 days).

Group 3: The continuous 17-β-estradiol group will receive the standard therapy for prevention of osteoporosis: A transdermal patch that releases 50ug/24 hrs of 17-β-estradiol will be administered continuously during the 16 weeks of treatment. The patch needs to be replaced twice a week (first patch is replaced after 4 days, second patch is replaced after 3 days).
See the SmPC for more information about the standard therapy.

*Cutting the patch in half will lead to administration of half the dose of the full patch, this is used off-label for treatment of menopausal symptoms (13). The 17-β-estradiol drug amount is uniformly distributed over the 17-β-estradiol matrix patch (14).

5.7 Preparation and labelling of Investigational Medicinal Product

Preparation and labelling will be performed by the Pharmacy of Amsterdam UMC, location AMC and will be done according to the GMP guidelines.

5.8 Drug accountability

A normal prescribing practice and documentation will be applied. Specific documentation of prescribed amounts and doses taken is available in the patients' medical records and in the eCRF.

Used patches will be returned to the investigator to assess compliance. After compliance has been reported in the eCRF, the used patches will be returned to the pharmacy to be destroyed.

6. NON-INVESTIGATIONAL PRODUCT

6.1 Name and description of non-investigational product(s)

The non-investigational auxiliary medical product (AxMP) will be administered to all participants in the trial, for endometrial protection.

Name: Micronized progesterone 100mg once daily
Trade name: Uterogestan
Formulation: capsule of 100mg

6.2 Summary of findings from non-clinical studies

The AxMP is authorised, and it will be used in the clinical trial in accordance with normal clinical practice as adjuvant therapy in combination with estradiol, to prevent endometrial hyperplasia. See SmPC for summary of findings (page 4-6).

6.3 Summary of findings from clinical studies

See SmPC (page 1-4)

6.4 Summary of known and potential risks and benefits

See SmPC (page 2-4)

6.5 Description and justification of route of administration and dosage

A structured risk analysis is given in Chapter 13.
The dosage and route of administration will be compliant to normal clinical practice from the Praktische handleiding hormoonsubstitutietherapie”” of the Dutch Menopause Society and Dutch Obstetrics and Gynaecology Society (NVOG)(13): continuous micronized progesterone 100mg once daily.

Participants will be instructed to take the medication in the evening before going to bed, as the medication can induce mild somnolence as a side effect during the first 1-4 hours of administration. Uterogestan is classified as a category 1 medical product according to the CBR (Centraal Bureau Rijvaardigheidsbewijzen): Participants will be informed on the side effects of progesterone and will be instructed not to drive if they experience somnolence, drowsiness or dizziness.

6.6 Dosages, dosage modifications and method of administration

Participants will receive progesterone 100mg once daily in the form of oral capsules, in accordance with the guidelines of the Dutch Menopause Society (DMS) and NVOG (13)

6.7 Preparation and labelling of Non Investigational Medicinal Product

Preparation and labelling will be performed by the Pharmacy of Amsterdam UMC, location AMC, conform the GMP guidelines

6.8 Drug accountability

A normal prescribing practice and documentation will be applied. Specific documentation of prescribed amounts and doses taken is available in the patients' medical records and in the eCRF.

Used capsule strips will be returned to the investigator to assess compliance. Any remaining capsules will be returned to the pharmacy to be destroyed.

7. METHODS

7.1 Study parameters/endpoints

7.1.1 Main study parameter/endpoint

Primary outcome: The primary outcome is the interaction between treatment and time on serum P1NP: Does the change over time differ between treatment arms? The primary endpoint will be measured every other week from baseline until week 16 (9 times in total).

Blood will be drawn every other week from baseline until week 16 (9 times in total) after an overnight fast.
P1NP measurements will be conducted by analysts in the Endocrinology Laboratory in the Amsterdam UMC. P1NP and will be measured using a non-competitive sandwich immunoassay (8% precision). Laboratory measurements of P1NP will all be performed in one batch for each participant.

7.1.2 Secondary study parameters/endpoints (if applicable)

Secondary outcome 1: The primary outcome is the interaction between treatment and time on serum CTX: Does the change over time differ between treatment arms? CTX will be measured every other week from baseline until week 16 (9 times in total).

Blood will be drawn every other week from baseline until week 16 (9 times in total)and all measurements will be measured at once in a batch for each participant.
CTX will be measured using non-competitive sandwich immunoassay (3.7% precision).

Secondary outcome 2: Difference between treatment arms in terms of change in fasting glucose, fasting insulin, insulin resistance (HOMA-IR), and post-OGTT outcomes from to baseline until 16 weeks of treatment.

Glucose and insulin will be measured in a fasting state and 2 hours after an 75g OGTT. These measurements will be performed at baseline and after 16 weeks. The analysis will be performed by analysts in the Endocrinology Lab and the Central Diagnostic Lab of the Amsterdam UMC.

7.1.3 Secondary outcome 3: Difference between treatment arms in terms of change in liver steatosis (CAP score), assessed with a fibroscan, from to baseline until 16 weeks of treatment.Other study parameters (if applicable)

Exploratory outcome 1: Difference between treatment arms in change of sleep quality (PSQI), chronotype (MCTQ) and menopause symptoms (GCS) scores from baseline until after 16 weeks of treatment.

Participants will fill in two sleep questionnaires (MCTQ, PSQI) and one menopausal symptoms questionnaire (GCS) at baseline and at week 16. The total scores from the questionnaires will be extracted and analysed.

Exploratory outcome 2: Effect of physiological vs. continuous 17-β-estradiol on 17-β-estradiol transcriptional regulation in adipose tissue (hypothesis-generating) using ChipSeq and RNA Seq analysis.

7.2 Randomisation, blinding and treatment allocation

If the subject is included in the trail, randomisation will be performed by the investigators using a validated block randomization model in CastorEDC with a block size of 4.

The study has an open-label design, due to the objective nature of the primary endpoints. Laboratory staff performing the laboratory measurements will be blinded to the treatment allocation, as the subject (pseudonymized ID) will not reveal the treatment allocation. P1NP and CTX measurements will be performed in one batch for each participant.

7.3 Study procedures

See the flow chart in section 2 for a schematic outline of the study procedures.

Screening: Patient history, family history, and medication use will be recorded, a physical examination will be performed (height, weight, blood pressure, waist circumference). The above mentioned data will be recorded in the eCRF (i.e. Castor EDC) as direct entry.
For screening purposes, fasting blood samples will be collected at baseline (4 tubes) to assess complete blood count, calcium, creatinine, ALAT, alkaline phosphatase, serum FSH, TSH, 25-hydroxyvitamin D (25(OH)D, glucose and HbA1c (%).

After inclusion, baseline measurements of fasting P1NP, CTX, estradiol, glucose and insulin will be conducted, and a 75g oral glucose tolerance test (OGTT) will be performed (4 tubes). A fibroscan (ultrasound) will be performed to measure baseline liver steatosis (CAP score). Plasma glucose and insulin will be measured at 2h after ingestion of the 75g glucose.

Subjects will additionally fill in two sleep questionnaires (MCTQ, PSQI) and menopause symptoms questionnaire (Greene Climacteric Scale) at baseline. The questionnaire data will be recorded in the eCRF (i.e. Castor EDC) as direct entry.

Treatment: Subjects will be allocated to one of the 3 groups and treated with transdermal estradiol patches for 16 weeks. The patch must be replaced twice a week (first patch after 4 days, second patch after 3 days). For a dose of 25µg/24hrs, the patch will be cut in half and applied to the skin.

During treatment: Subjects will undergo venepuncture at baseline and every other week (window ±1 days) for the duration of the treatment (9 times in total). At each venepuncture visit, 1 tube of blood will be drawn.

At week 8 (window ±1 day) and week 16 (window ±1 day): participants will visit the hospital for a visit with the investigator. The investigator will measure the participants' blood pressure, report any new medications that the subject has been taking and the investigator will report any adverse events that occurred. At week 16, extra blood will be drawn (3 tubes) to measure fasting glucose, fasting insulin and an 75g oral glucose tolerance test (OGTT) will be performed. A fibroscan (ultrasound) will be performed to measure post-treatment liver steatosis (CAP score). Plasma glucose and insulin will be measured at 2h after ingestion of the 75g glucose. The participant will also fill in two sleep and menopause questionnaires (MCTQ, PSQI, GCS) at week 16.

Optional: Participants will optionally undergo two subcutaneous adipose tissues biopsies, at baseline and at week 16. Adipose tissue biopsies will be obtained by vacuum suction as described in (Stenvers et al. Diabetologia, 2019)(16). After local anaesthesia with lidocaine, these superficial subcutaneous adipose tissue samples are obtained by vacuum suction with a 15 gauge needle, from a peri-umbilical

quadrant. All samples will be stored at -80 degrees Celsius as soon as possible after sampling (16). RNA sequencing will be performed as described previously (16).

7.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

7.4.1 Specific criteria for withdrawal (if applicable)

The IMP will be discontinued immediately when one or more of the following events occur:

- Venous thromboembolism
- Significant increase of blood pressure: ≥30 mmHg systolic and/or ≥15 diastolic compared to baseline OR blood pressure ≥160 mmHg systolic and ≥110mmHg diastolic
- Jaundice
- Severe (migrainous) headache (in patients without history of headaches/migraines)

In case of (cumbersome) side-effects of the treatment, the investigator and the participant will assess whether the participant should discontinue treatment.

7.5 Replacement of individual subjects after withdrawal

Subjects who withdraw from the study will be replaced.

7.6 Follow-up of subjects withdrawn from treatment

The investigator will contact the subject for a safety check one week after the study treatment has finished (or one week after the subjects have withdrawn from treatment). If subjects experience abnormal vaginal bleeding, they will be referred to the GP. Subjects will be instructed to contact a GP if abnormal vaginal bleeding occurs after the trial.

7.7 Premature termination of the study

See section 8 of the protocol.

8 SAFETY REPORTING

8 Temporary halt for reasons of subject safety

In accordance with section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

8.1 AEs, SAEs and SUSARs

8.1.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product / trial procedure. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded in the eCRF. The severity is graded according to the grading criteria of the National Institutes of Health NCI Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0.

8.1.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death
- is life threatening (at the time of the event)
- requires hospitalisation or prolongation of existing inpatients' hospitalisation
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.
-

The following situations do <u>not</u> need to be reported as SAEs:

- Admission for diagnosis or therapy of a condition that existed before the start of the study **and** has not increased in severity or frequency as judged by the clinical investigator.
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present at the start of the study that do not worsen.

- Any admission unrelated to an AE, e.g., for labour/delivery, cosmetic surgery, social and/or convenience admissions to a hospital.
- Elective hospitalisation (planned before the subject consented for study participation) for pre-existing conditions that did not exacerbate during the study period as judged by the clinical investigator and where admission did not take longer than anticipated.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

8.1.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2)
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
   - Summary of Product Characteristics (SPC) for an authorised medicinal product
   - Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC.

- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.
The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life-threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

8.2 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report combined with the annual progress report to the accredited METC, competent authority, and competent authorities of the concerned Member States.
This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study.
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

8.3 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

8.4 Data Safety Monitoring Board (DSMB)

Not applicable, as the risk of the study is low. The investigational product is registered for the studied indication and will be administered in the standard dose or a lower dose. Furthermore, the risk of complications when using transdermal 17-β-estradiol or progesterone is very low.

9 STATISTICAL ANALYSIS

Data will be presented quantitatively. Patients are evaluable if the primary outcome is available for each week (either directly or through imputation). Statistical analyses will be carried out in SPSS and all statistical significance will be set at a two-sided p-value of 0.05.

We will include subjects in the analysis conform a per-protocol analysis to explore efficacy of rhythmic transdermal 17-β-estradiol vs. low-dose and standard-dose continuous transdermal 17-β-estradiol.

An exploratory intention-to-treat analysis will be performed to explore the clinical effectiveness of the treatment and to assess if rhythmic transdermal 17-β-estradiol leads to reduced compliance compared with continuous transdermal 17-β-estradiol.

9.1 Primary study parameter(s)

The primary outcome is the interaction between treatment and time on serum P1NP concentrations. The primary endpoint will be measured every other week from baseline until week 16 (9 times in total).

The difference between arms in terms of change over time in the primary outcome measure (P1NP) will be analysed using a mixed model for repeated measures. As fixed effects, the model will include the main and interaction effects of treatment and time, adjusted for age, years since last menstruation and BMI. In addition, random patient intercept and/or slope effects will be included.

Strategy for multiple testing correction:

1) Compare the model fit of the models with/without the treatment*time interaction effect using a likelihood ratio test, and in case of a significant ($p<0.05$) improvement

2) Evaluate which interaction effects are significant. The significance threshold for these pairwise comparisons will be corrected for the number of comparisons made.

Additionally, the mean bone marker response after 16 weeks compared to baseline will be compared between groups using a one-way ANOVA or Kruskall-Wallis tests.

9.2 Secondary study parameter(s)

The secondary outcome is the interaction between treatment and time on serum CTX concentrations. The difference between arms in terms of change over time in the secondary outcome measure (CTX) will be analysed using a mixed model for repeated measures. As fixed effects, the model will include the main and interaction effects of treatment and time, adjusted for age, years since last menstruation and BMI. In addition, random patient intercept and/or slope effects will be included.

Additionally, the mean bone marker response after 16 weeks compared to baseline will be compared between groups using a one-way ANOVA or Kruskall-Wallis tests.

Change from baseline of fasting glucose, fasting insulin, HOMA-IR, C-peptide and 75g OGTT measurements and CAP score change from baseline will be compared between groups using a ANOVA tests or Kruskall-Wallis test depending on the distribution.

9.3 Other study parameters

Sleep questionnaires and menopause questionnaires (MCTQ, PSQI, GCS) will be analysed by comparing change from baseline scores between groups using an ANOVA test or Kruskall-Wallis test depending on the distribution.

Subcutaneous fat biopsies: RNA sequencing and Chip sequencing will be analysed using validated techniques.

9.4 Interim analysis (if applicable)

Not applicable

10 ETHICAL CONSIDERATIONS

10.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (version October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO), Geneesmiddelenwet, Clinical Trials Directive, AVG, WGBO and ICH GCP E6(R2) Richtsnoer.

10.2 Recruitment and consent

Subjects will be recruited through advertisements in the hospital, on social media, on recruitment websites and through advertisements in GP practices.

The potential participant will have at least 10 days to decide whether or not they want to participate in the trial.

Informed consent will be obtained prior to any study related procedures being undertaken at screening. Informed consent will be written, dated, and signed by the person performing the interview, and by the subject. The investigator or his/her representative will explain the nature of the study to the subject and answer all questions regarding this study. In the interview it will be verified that the subject has understood the information. The subject will be provided with a copy of the document (or the record) by which informed consent has been given. The informed consent will be documented. Adequate time will be given for the subject to consider his or her decision to participate in the clinical trial.

10.3 Objection by minors or incapacitated subjects (if applicable)

Not applicable

10.4 Benefits and risks assessment, group relatedness

Benefits: This study will provide new insight on the effects of rhythmic administration of estrogen on bone turnover. This study will explore whether low-dose rhythmic 17-β-estradiol administration has the potential to improve treatment for osteoporosis prevention. Participation in the trial does not involve an expected individual benefit for the participant other than relieving possible climacteric symptoms. However, there might be a group benefit if physiological low-dose estrogen therapy leads to increased bone turnover and improved treatment for the prevention of osteoporosis in postmenopausal women. Osteoporosis is particularly prevalent in postmenopausal women. Osteoporotic fragility

fractures lead to high morbidity rate, endanger patients' health and interferes with their quality of life (17). Thus, prevention of osteoporosis is crucial to improve mental and physical health in postmenopausal women.

Risks: Blood draws may cause bruising at the site of the punctured vein. Participants may experience side effects of the transdermal 17-β-estradiol treatment. The most common side effects are rash at the site of the transdermal patch (20.8%), redness at the site of the patch (8.5%), itchiness at the site of the patch (19.8%), headache (7.8%), breast tenderness (6.6%). The auxiliary medical product may cause the following side effects: vaginal bleeding, breast tenderness, nausea, dizzeness, somnolence, headache (SmPC). Combined estrogen with progesterone therapy does not lead to an increased risk of endometrial hyperplasia and endometrial cancer (18). Evidence shows that there is no excess risk of venous thromboembolism in transdermal 17-β-estradiol users (6). Hormone replacement therapy for less than 1 year does not lead to increased risk of breast cancer (7)(SmPC).
The questionnaires take some time to answer.

Optionally, participants will undergo two subcutaneous fat biopsies: One fat biopsy at baseline and one after 16 weeks. Participants will experience discomfort during the application of local anaesthesia, but thereafter the procedure is painless. A subcutaneous hematoma will develop after the fat biopsy, but this will gradually resolve. The subcutaneous fat biopsy can possibly cause bleeding or an infection around the site of the biopsy.

10.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.
The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

10.6 Incentives (if applicable)

Subjects will receive reimbursement for their travel expenses.

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Handling and storage of data and documents

The study will be conducted in compliance with the protocol and with the principles of good clinical practice. The data will be collected and processed in accordance with the General Data Protection Regulation (EU) 2016/679.

Data will be stored in a secure folder on the G:\ disc of the department, this folder is only accessible for the investigators. Personal data will be pseudonymized using a unique subject identification code in accordance with the GDPR. The key to the code will be kept in a secured SPSS file with a password only known by the investigators and monitors. Pseudonymized data will be stored in a Castor eCRF and extracted to SPSS. Participants will fill in the questionnaires in an online Castor survey. Patient data will be kept up to 15 years in accordance with the FMWV code of conduct for responsible use.
In case of a security breach, the privacy officer will be notified immediately.
Data will not be transferred to countries outside the EU.

Body material (blood) will be stored at the Laboratory of Endocrinology until further processing. Body material will be used for predefined study outcomes mentioned in this protocol. Any remaining body material will be destroyed after all processing has been completed and within 5 years after collection of the body material. The body material will be coded using the subject identification code and the study name. The key to the code will be kept in a secured SPSS file with a password only known by the investigators and monitors.

11.2 Monitoring and Quality Assurance

The clinical monitoring centre (CMC) of the Amsterdam UMC, location AMC, will monitor the present study. The risk for participating in the study is low. The monitoring plan will be constructed with a clinical research associate (CRA) of the clinical monitoring centre (CMC) after this protocol has been approved.

11.3 Amendments

A ‘substantial amendment’ is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial.
- the scientific value of the trial.

- the conduct or management of the trial, or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority but will be recorded and filed by the sponsor.

11.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

11.5 Temporary halt and (prematurely) end of study report

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

11.6 Public disclosure and publication policy

The trial will be registered a trial register (e.g. clinicaltrials.gov or Netherlands Trial Register) prior to the start of the study.
The results will be submitted for publication in a peer-reviewed medical journal.

12. STRUCTURED RISK ANALYSIS

12.1 Potential issues of concern

The investigational study product (transdermal 17-β-estradiol) combined with micronized progesterone has been studied extensively and is a registered treatment for the prevention of osteoporosis. Details on mechanism of action, effects, and interactions are listed in the SmPC.

Therefore, chapter 12.1 is skipped.

12.2 Synthesis

12.2.1. Transdermal 17-β-estradiol

Risks that have been associated with (transdermal) 17-β-estradiol use are: Development of venous thromboembolism, endometrial hyperplasia, breast cancer, endometrial cancer, ovarian cancer, coronary heart disease and cerebrovascular disease (SmPC).

Risk synthesis:

Venous thromboembolism (VTE): The risk of VTE is elevated when oral estrogen preparations are used, however, evidence demonstrates that transdermal estrogen therapy does not increase the risk of VTE (Richtlijn Antitrombotisch Beleid 2020, (19)). A recent meta-analysis found that transdermal estrogen users have a similar risk of VTE compared to non-users of estrogen. Other meta-analyses confirm that the risk of VTE is not elevated when estrogen is administered transdermally (20-22).

Endometrial hyperplasia and endometrial cancer:

Combined HRT (estrogen + progestogen), is not associated with an increased risk of endometrial hyperplasia and endometrial cancer (SmPC).

Breast cancer:

The risk of breast cancer because of estrogen replacement therapy depends on the treatment duration. There is no excess risk for breast cancer when estradiol + micronized progesterone is used for less than 1 year (7, 19).

Ovarian cancer:

Estrogen replacement is associated with a low increased risk of ovarian cancer. After five years of estrogen use, the absolute increased risk of ovarian cancer is 0.05% (SmPC).

Cerebrovascular disease and coronary heart disease:

The risk of coronary heart disease is slightly elevated in HRT users aged >60 years (SmPC). Recent prospective studies suggest that transdermal estrogen therapy (≤50ug/24hrs) does not elevate stroke risk (23, 24).

Other rare complications of estrogen replacement therapy are listed in the SmPC.

The following measures to prevent/reduce the risk of the above-described complications will be outlined separately for each complication:

Venous thromboembolism: 17-β-estradiol will be administered through a transdermal patch, which does not increase the risk of thromboembolism.
Participants with a history of venous thromboembolism (VTE), known genetic or inherited thrombophilia, liver disease, abnormal liver enzymes, any other medical condition and use of any medication known to increase the risk of VTE will be excluded from the study.
Participants will be instructed to immediately consult their GP if they notice painful swelling and/or redness of a leg or arm. Participants will be instructed to consult their GP or the emergency services if they notice dyspnea or chest pain.

Coronary heart disease: Participants aged >60 years will be excluded from the study.
Participants will be excluded if they have a history of myocardial infarction, recent angina pectoris or history of angina pectoris, and/or uncontrolled hypertension (systolic: ≥140 mmHg, diastolic ≥90 mmHg in two separate measurements).
Participants will be instructed to consult their GP or the emergency services if they experience severe dyspnea or chest pain.

Cerebrovascular disease: Participants will receive low dose (50ug/24hrs) and/or very low dose (25ug/24hrs) transdermal estrogen therapy. Participants with a known history of cerebrovascular disease, including transient ischemic attack (TIA), will be excluded.

Endometrial hyperplasia: We will exclude participants with abnormal vaginal bleeding and known untreated endometrial hyperplasia.
Subjects will be treated with oral natural progesterone 100mg once daily, to reduce the risk of endometrial hyperplasia.

Breast cancer: Participants with a history, suspicion, or presence of breast cancer will be excluded.

Ovarian cancer: Participants with a history or presence of ovarian cancer will be excluded.

Due to the above-described safety measures, the risk for subjects participating in the study is low.

12.2.2. Progesterone

Micronized progesterone (natural progesterone) is a registered product used to prevent endometrial hyperplasia. The brand name is Uterogestan, and the recommended dose in combination with 50 microgram transdermal estradiol is 100mg daily (Praktische handleiding hormoonsubstitutietherapie Dutch Menopause Society &NVOG, 2020)(13). In the present study, uterogestan will be used as an auxiliary medical product.

Short summary of the risk listed in the SmPC, and measures taken to reduce these risks:
Micronized progesterone is contra-indicated if there is presence of a liver disease, liver tumors, Rotor-syndrome, and Dubin-Johnson syndrome. Participants with presence of the above contra-indications will be excluded from the trial.

Micronized progesterone may mask abnormal vaginal bleeding. Therefore, abnormal vaginal bleeding is an exclusion criterium for the study.

An uterogestan capsule contains soyecithin, which in rare cases may cause anaphylactic shock. There is an association between soy allergy and peanut allergy and the occurrence of anaphylactic shock due to soyecithin. Participants with a soy allergy or peanut allergy will be excluded from the trial.

Possible side effects of micronized progesterone include vaginal bleeding or “spotting”, breast tenderness, nausea, vertigo, headache, somnolence. See the SmPC for all side effects. Participants will be instructed to take oral progesterone in the evening before going to sleep, as progesterone may cause somnolence.

When used in accordance with the correct dosage, micronized progesterone does not lead to an increased risk of cardiovascular disease (SmPC).

13. REFERENCES

1. Manolagas SC. From estrogen-centric to aging and oxidative stress: a revised perspective of the pathogenesis of osteoporosis. Endocr Rev. 2010;31(3):266-300.

2. Lindsay R, Hart DM, Forrest C, Baird C. Prevention of spinal osteoporosis in oophorectomised women. Lancet. 1980;2(8205):1151-4.
3. Vatrasresth J, Suwan A, Panyakhamlerd K. Effects of early estradiol valerate administration on bone turnover markers in surgically induced menopausal women. BMC Womens Health. 2021;21(1):363.
4. Mauvais-Jarvis F, Clegg DJ, Hevener AL. The role of estrogens in control of energy balance and glucose homeostasis. Endocr Rev. 2013;34(3):309-38.
5. Mauvais-Jarvis F, Manson JE, Stevenson JC, Fonseca VA. Menopausal Hormone Therapy and Type 2 Diabetes Prevention: Evidence, Mechanisms, and Clinical Implications. Endocr Rev. 2017;38(3):173-88.
6. Rovinski D, Ramos RB, Fighera TM, Casanova GK, Spritzer PM. Risk of venous thromboembolism events in postmenopausal women using oral versus non-oral hormone therapy: A systematic review and meta-analysis. Thromb Res. 2018;168:83-95.
7. Collaborative Group on Hormonal Factors in Breast C. Type and timing of menopausal hormone therapy and breast cancer risk: individual participant meta-analysis of the worldwide epidemiological evidence. Lancet. 2019;394(10204):1159-68.
8. Hannon R, Blumsohn A, Naylor K, Eastell R. Response of biochemical markers of bone turnover to hormone replacement therapy: impact of biological variability. J Bone Miner Res. 1998;13(7):1124-33.
9. Limonard EJ, Veldhuis-Vlug AG, van Dussen L, Runge JH, Tanck MW, Endert E, et al. Short-Term Effect of Estrogen on Human Bone Marrow Fat. J Bone Miner Res. 2015;30(11):2058-66.
10. Crofton PM, Evans N, Bath LE, Warner P, Whitehead TJ, Critchley HO, et al. Physiological versus standard sex steroid replacement in young women with premature ovarian failure: effects on bone mass acquisition and turnover. Clin Endocrinol (Oxf). 2010;73(6):707-14.
11. Salpeter SR, Walsh JM, Ormiston TM, Greyber E, Buckley NS, Salpeter EE. Meta-analysis: effect of hormone-replacement therapy on components of the metabolic syndrome in postmenopausal women. Diabetes Obes Metab. 2006;8(5):538-54.
12. Gezondheidsraad. Evaluatie van de voedingsnormen voor vitamine D. Den Haag: Gezondheidsraad; 2012.
13. Brood-van Zanten MMAvT, M.M.A.; van Dijken, D.K.E.; Janssen, C.A.H. Menopauze: Praktische handleiding hormoonsuppletietherapie (HST). Richtlijnwerkgroep Menopauze Management van de Nederlandse Vereniging voor Obstetrie en Gynaecologie (NVOG); 2020.
14. Ankarberg-Lindgren C, Gawlik A, Kristrom B, Mazzanti L, Ruijgrok EJ, Sas TCJ. Estradiol matrix patches for pubertal induction: stability of cut pieces at different temperatures. Endocr Connect. 2019;8(4):360-6.
15. Stanosz S, Zochowska E, Safranow K, Sieja K, Stanosz M. Influence of modified transdermal hormone replacement therapy on the concentrations of hormones, growth factors, and bone mineral density in women with osteopenia. Metabolism. 2009;58(1):1-7.
16. Stenvers DJ, Jongejan A, Atiqi S, Vreijling JP, Limonard EJ, Endert E, et al. Diurnal rhythms in the white adipose tissue transcriptome are disturbed in obese individuals with type 2 diabetes compared with lean control individuals. Diabetologia. 2019;62(4):704-16.
17. Chinese Orthopaedic A. Diagnosis and treatment of osteoporotic fractures. Orthop Surg. 2009;1(4):251-7.
18. Furness S, Roberts H, Marjoribanks J, Lethaby A. Hormone therapy in postmenopausal women and risk of endometrial hyperplasia. Cochrane Database Syst Rev. 2012;2012(8):Cd000402.
19. NVOG. Richtlijn management rondom menopauze. 2018.
20. Canonico M, Fournier A, Carcaillon L, Olie V, Plu-Bureau G, Oger E, et al. Postmenopausal hormone therapy and risk of idiopathic venous thromboembolism: results from the E3N cohort study. Arterioscler Thromb Vasc Biol. 2010;30(2):340-5.
21. Renoux C, Dell'Aniello S, Suissa S. Hormone replacement therapy and the risk of venous thromboembolism: a population-based study. J Thromb Haemost. 2010;8(5):979-86.

22. Mohammed K, Abu Dabrh AM, Benkhadra K, Al Nofal A, Carranza Leon BG, Prokop LJ, et al. Oral vs Transdermal Estrogen Therapy and Vascular Events: A Systematic Review and Meta-Analysis. J Clin Endocrinol Metab. 2015;100(11):4012-20.
23. Lokkegaard. Risk of Stroke With Various Types of Menopausal Hormone Therapies: A National Cohort Study (vol 48, pg 2266, 2017). Stroke. 2018;49(3):E142-E.
24. Renoux C, Dell'aniello S, Garbe E, Suissa S. Transdermal and oral hormone replacement therapy and the risk of stroke: a nested case-control study. BMJ. 2010;340:c2519.