STUDY TITLE

Optimizing metformin use in polycystic ovary syndrome – A randomized controlled trial

REFERENCE NUMBERS

EU trial number: 2023-509259-15-01
Date and version number: 15th January 2025, 5. version

STUDY SPONSOR

HUS-yhtymä

COMPLIANCE STATEMENT

This trial is to be conducted in compliance with this protocol, with the Regulation 536/2014 of the European Parliament and of the Council and with the principles of Good Clinical Practice.

CONFIDENTIALITY STATEMENT

This document contains confidential information that must not be disclosed to anyone other than the sponsor, the study team, regulatory authorities, and members of the Ethics Committee.

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1 SYNOPSIS

Title of study	Optimizing metformin use in polycystic ovary syndrome – A randomized controlled trial
Name of sponsor/company	Helsinki University Hospital
Aim	To evaluate if a metformin dose of 1500 mg per day is inferior to 2250 mg per day in improving biochemical and clinical outcomes, as well as side-effects, mental health and quality of life in individuals 18-37 years of age with PCOS and a BMI ≥ 25 and <40 kg/m2
Trial design	A double blinded randomized controlled trial
Key inclusion criteria	Individuals with PCOS (diagnosed according to the International evidence-based Guideline for PCOS 2023) Age: 18-37 years BMI: ≥ 25 and <40 kg/m2
Key exclusion criteria	-Individuals not meeting the criteria according to the International evidence-based Guideline for PCOS 2023) -Individuals with hormonal contraceptive during the last 3 months -Individuals that are pregnant or breastfeeding -Individuals with an untreated diabetes, hypothyroidism or hyperprolactinemia -Use of medications for diabetes, high cholesterol, obesity and cortisone (per oral) -Individuals with contraindication for use of metformin (hypersensitivity to metformin, acute metabolic acidosis, renal impairment, hepatic insufficiency, heart- or respiratory failure) -Serious mental illness. -Alcoholism - Investigator site staff directly involved in the conduct of the study and their family members
Test product, dose and mode of administration	Metformin 500 mg x3 /day orally Metformin 750 mg x3/day orally
Duration of treatment	26 weeks (first two weeks with reduced dose)
Statistical methods	We will summarise the outcomes by number of patients, mean, standard deviation, median, minimum, and maximum values and report mean differences. We will follow the intention-to-treat principle and adjust/perform sub-analyses according to age, BMI and PCOS phenotype. Primary analysis: For normally distributed variables, independent-sample t-tests will be used to compare values between and within the two treatment groups at

	each time point (baseline, 14 and 26 weeks). Mann-Whitney will be performed for variables with skewed distribution. Supplementary analysis: Repeated measures ANOVA will be performed to estimate the within-group modifications. We will also use linear mixed-effects models when comparing the two metformin doses regarding change from baseline measurement and when comparing the two groups to each other.
Sample size	184 individuals (92 in each group)

2 ABBREVIATIONS AND DEFINITION OF TERMS

BMI	Body Mass Index
PCOS	Polycystic Ovary Syndrome
WHR	waist-hip ratio
WC	Waist circumference
BAI	Body adiposity index
BP	blood pressure
FAI	free androgen index
SHBG	sex hormone binding globulin
DHEAS	dehydroepiandrosterone sulfate
HOMA-IR	Homeostatic model assessment for insulin resistance
CRP	C-reactive protein
FLI	Fatty Liver Index
ALAT	Alanine aminotransferase
GGT	Gamma-glutamyltransferase
QoL	Quality of life
GI AE	Gastrointestinal adverse effects
AMH	anti-mullerian hormone
FNPO	Follicle number per ovary
TSH	Thyroid stimulating hormone
PRL	Prolactin
17-OHP	17-hydroxyprogesterone
GFR	Glomerular filtration rate
OD	Ovulatory dysfunction
HA	Hyperandrogenism
PCOM	Polycystic ovary morphology
TyG	Triglyceride glucose
RSI	Reference safety information
CTIS	Clinical Trials Information System
CRF	Case report form
SAE	Serious adverse event
SUSAR	Suspected unexpected serious adverse reaction
GCP	Good Clinical Practice

3 INTRODUCTION

Polycystic ovary syndrome (PCOS) remains a key public health burden as it is one of the most common endocrine and metabolic disorders affecting up to 13% of women globally (1). The Rotterdam diagnostic criteria were updated and internationally endorsed in the 2023 International PCOS guideline and include a) ovulatory and menstrual dysfunction, b) biochemical and/or clinical hyperandrogenism and c) polycystic ovary morphology (PCOM) at ultra-sound. For a diagnosis in adults two of three criteria are required, with PCOM remaining nonspecific for PCOS in adolescents (2, 3).

3.1 Background information

Women with PCOS present with diverse features; metabolic (weight gain, obesity, insulin resistance and diabetes) reproductive (infertility and pregnancy complications), endocrine (hyperandrogenism, hirsutism and acne) and psychosocial (depression, anxiety and poor quality of life) (3, 4).

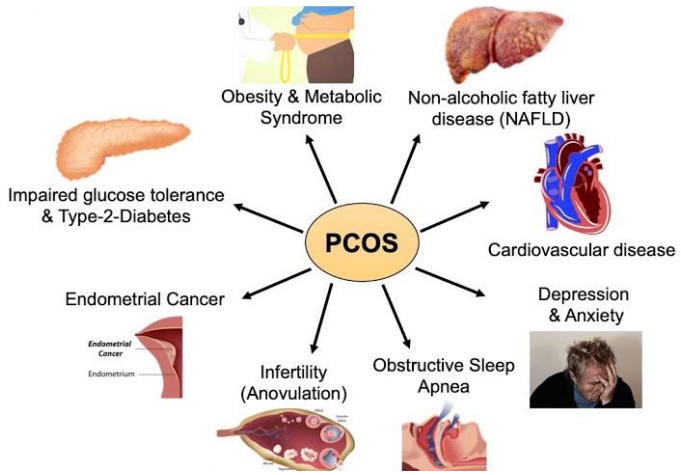


Figure of the most common features of PCOS

While lifestyle management is strongly recommended for weight management (2, 5), for those with higher BMI, sustainable efficacy for weight loss can be limited and additional pharmacological treatment may be needed. The 2023 Updated International PCOS Guideline recommends that metformin, in addition to lifestyle, should be considered in adults with PCOS and BMI ≥ 25 kg/m2 for prevention of weight gain and management of weight and metabolic disorders. Metformin may be considered in adults with BMI < 25 kg/m2 and adolescents with PCOS, acknowledging more limited evidence (6).

3.2 Rationale for the study

MD Johanna Melin has been working on the 2023 update of the International Evidence-based PCOS Guideline as the team leader of the clinical question "is metformin alone or in

combination, effective for management of hormonal and clinical PCOS features and weight in adolescents and adult with PCOS". During her work with the PCOS guideline she has identified the following key knowledge gaps:

a) the optimal metformin dose for adults with PCOS is unclear regarding metabolic outcomes, hyperandrogenism, cycle regularity and side-effects.
b) we lack information on mental health and quality of life in women with PCOS and if they can be improved by medical treatment with metformin
c) we lack information on whether anti-mullerian hormone (AMH) levels will change after use of metformin.

4 STUDY OBJECTIVE

The aim of this randomized controlled trial (RCT) is to evaluate whether a metformin dose of 1500 is inferior compared to a dose of 2250 mg per day in improving biochemical and clinical outcomes, as well as side-effects, mental health and quality of life in females 18-37 years of age with PCOS.

During the rigorous systematic review comparing the effect of metformin to other medical treatments in the updated 2023 PCOS guidelines, only one small study compared different metformin doses. This is a highly relevant question, as side-effects are a common reason for discontinuing the metformin treatment and they will increase with higher metformin doses.

Our hypothesis is that individuals using the lower metformin dose might improve their biochemical (hyperandrogenism, insulin resistance, lipids etc) and clinical outcomes (hirsutism and weight) to the same extent than those using a higher metformin dose.

4.1 Primary objective

Altogether six publications with six primary objectives are planned from this RCT:

1. to evaluate whether a metformin dose of 1500 mg per day is inferior to a dose of 2250 mg per day in improving **anthropometric outcomes** in women with PCOS
2. to evaluate whether a metformin dose of 1500 mg per day is inferior to a dose of 2250 mg per day in improving **metabolic outcomes** in women with PCOS
3. to evaluate whether a metformin dose of 1500 mg per day is inferior to a dose of 2250 mg per day in improving **hyperandrogenism** in women with PCOS
4. to evaluate whether metformin at a dose of 1500 mg per day is inferior to a dose of 2250 mg per day regarding gastrointestinal **side-effects** in women with PCOS
5. to evaluate whether a metformin dose of 1500 mg per day is inferior to a dose of 2250 mg per day in improving **quality of life/mental health and weight stigma** in women with PCOS
6. to evaluate whether a metformin dose of 1500 mg per day is inferior to a dose of 2250 mg per day in improving **polycystic ovary morphology and menstrual cyclicity** in women with PCOS**.**

4.2 Primary and secondary/exploratory endpoints/outcome measures

Primary outcomes below are bolded, whereas all not bolded are secondary endpoints.

Anthropometric:

- **Weight**. Primary outcome. Change in weight (kg) from baseline to 14 and 26 weeks.
- BMI (Body Mass Index). Change in BMI (kg/m2) from baseline to 14 and 26 weeks
- WC (waist circumference. Change in WC from baseline to 14 and 26 weeks
- WHR (Waist-hip-ratio). Change in WHR from baseline to 14 and 26 weeks
- BAI (Body adiposity Index). Change in BAI from baseline to 14 and 26 weeks
- Body composition measurement. Change in body composition from baseline to 14 and 26 weeks
- BP (bloodpressure) (systolic and diastolic) and pulse. Change in BP (mmHg) from baseline to 14 and 26 weeks

Metabolic:

For all the below mentioned laboratory tests we measure change from baseline to 14 and 26 weeks

- **HOMA-IR** (Homeostatic model assessment for insulin resistance). Primary outcome
- fasting glucose
- fasting insulin
- HbA1C
- Lipids (cholesterol and its subclasses, triglycerides)
- CRP (C-reactive protein)
- 2 hour OGTT (oral glucose tolerance test)
- ALAT (alanine aminotransferase)
- G-GT (gamma-glutamyltransferase).
  From these tests, following indexes can be calculated:
  FLI (fatty liver index (FLI)
  Matsuda Index (indicator for insulin resistance)
  Triglyceride glucose (TyG) index (indicator for insulin resistance)

Androgenicity:

Clinical:

- **Hirsutism**. Primary outcome. Here we measure change in modified Ferriman-Gallwey score from baseline to 14 and 26 weeks
- Acne
- Alopecia

Biochemical:

For all the below mentioned laboratory tests we measure change from baseline to 14 and 26 weeks

- **Testosterone.** Primary outcome.
- FAI (free androgen index)

- SHBG (sex hormone binding globulin)
- DHEAS (dehydroepiandrosterone sulfate)
- Androstenedione
- free testosterone

Menstrual Cyclicity:

- Questionnaire, where asking if the menstrual cycle is regular (defined as 21 days or more but under 36 days) and measuring change from baseline to 14 and 26 weeks.
- Questionnaire, where asking how long the two previous cycles were (days) and measuring change from baseline to 14 and 26 weeks

Gastrointestinal side-effects:

- **Questionnaire,** where asking about the appearance and severeness of the following symptoms: abdominal pain, reflux, nausea, diarrhoea, loss of appetite and measuring change from baseline to 14 and 26 weeks. Primary outcome will be severeness of gastrointestinal adverse effects (GI AE). A severe adverse effect is defined as a score of 4 or more in one of the questions in the questionnaire (indicating a daily experience of abdominal pain, reflux, nausea, diarrhoea or loss of appetite during the last two weeks). Secondary outcomes will be appearance of GI AE (never/everything else but never), appearance of different AE (abdominal pain, reflux, nausea, diarrhoea, loss of appetite) and discontinuation of metformin due to GI AE.

Quality of life and weight stigma:

- The PCOS-Q- a questionnaire that was specifically designed to measure quality of life in women with PCOS.

Polycystic ovary morphology:

- **AMH** (anti-Mullerian hormone). Here we measure change from baseline to 14 and 26 weeks. Primary outcome.
- Vaginal ultrasound where we measure follicle number per ovary and ovarian volume and change from baseline to 14 and 26 weeks.

5 TRIAL DESIGN

5.1 General considerations

The present study is a double-blind, randomized controlled trial, including adults (age 18-37 years) with PCOS and a BMI ≥ 25 and <40 kg/m2. Potential participants for the study will be recruited from individuals referred to the Reproductive Medicine Unit at Helsinki University Hospital in Finland and by advertising via the Finnish patient organization for those with PCOS

(Korento). Medicine use included and excluded in this study are listed in Table 1. The PICO (Population, Intervention, Comparison and Outcome) frameworks for this RCT have been established *a priori* and will be used for study selection (Table 2).

5.1.1 Overall description of trial subjects

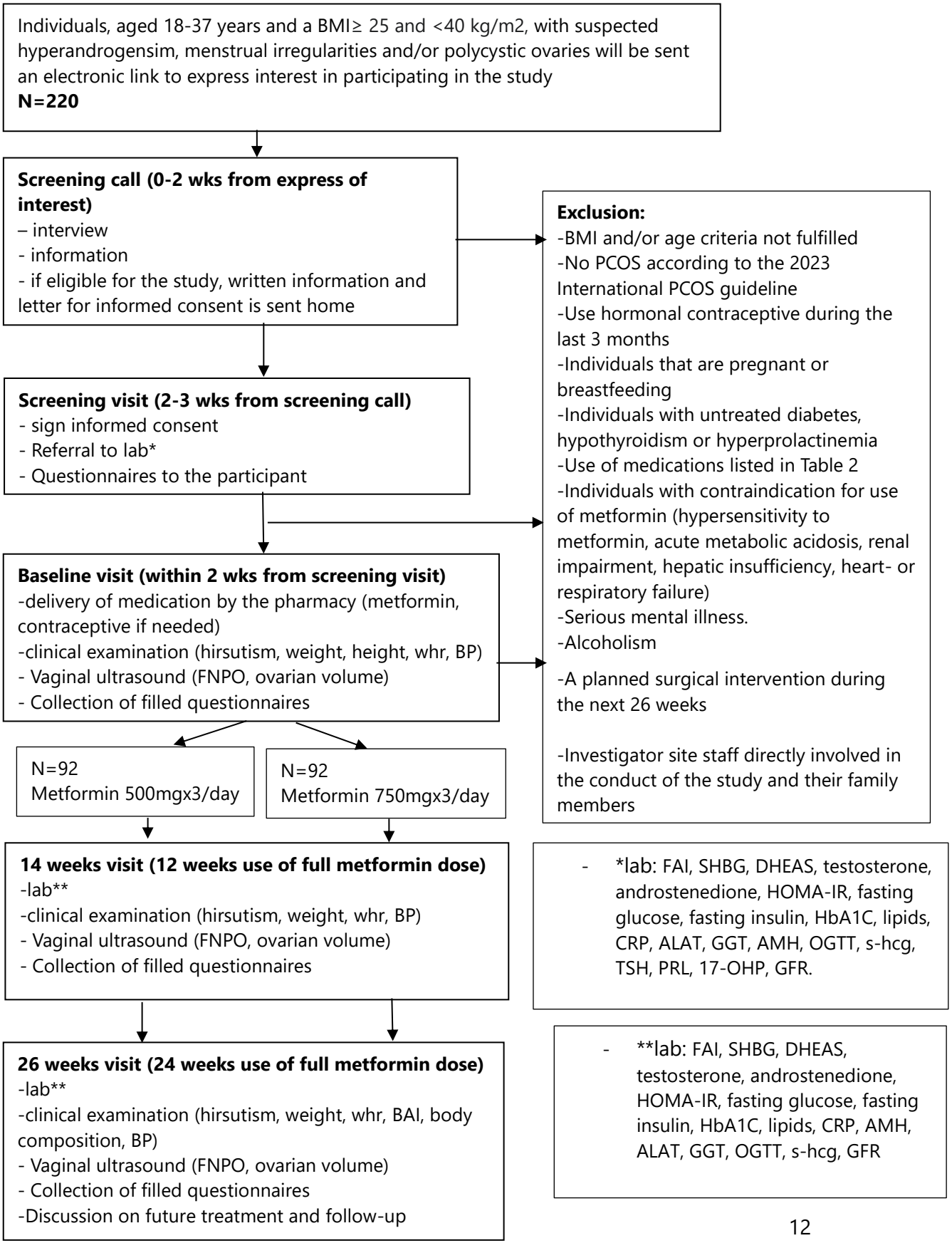
The 2023 updated international evidence-based guideline for the assessment and management of PCOS recommends using the Rotterdam criteria for the diagnosis of PCOS (3). For a PCOS diagnosis in adults, two out of three following criteria are required: ovulatory dysfunction (OD); clinical or biochemical hyperandrogenism (HA); and/or polycystic ovary morphology (PCOM). In addition, other causes, for example hypothyroidism and hyperprolactinemia, should be excluded.

Adolescents have other diagnostic criteria for PCOS than adults, as menstrual irregularities and PCOM are much more common in this age category, which is why we have excluded them from this study.

Ovarian dysfunction (OD)

The OD criterium indicates oligo-or anovulation, with irregular menstrual cycles as the most common symptom. In the updated 2023 PCOS guideline, criteria for OD are defined as a menstrual cycle < 21 or > 35 days, three years after menarche until perimenopause.

Figure 1: Study Schema



Hyperandrogenism (HA)

HA can be assessed either clinically or biochemically. Regarding clinical HA, hirsutism is highly correlated with biochemical HA whereas acne and alopecia are less specific. For objective assessment of hirsutism, the use of the modified Ferriman-Gallwey (mFG) score is recommended, recognizing that self-treatment is frequently employed. For hirsutism, a mFG score of 4 or more is required in Caucasian women. If clinical HA is not present, biochemical hyperandrogenism can be determined. Biochemical HA is best assessed using total testosterone or free androgen index (FAI) analysed with highly accurate tandem mass spectrometry (LC-MS/MS) assays. A testosterone of 2,0 nmol/l or higher or a FAI of 6 or higher is regarded as biochemical hyperandrogenism.

Polycystic ovary morphology (PCOM)

PCOM can only be assessed when over 8 years has passed from the menarche. The assessment of PCOM has until recently been done by ultrasonography but in the 2023 international PCOS guideline, measurement of serum anti-Müllerian hormone (AMH) has been added as an alternative. Ultrasound is at present still the primary method for assessing PCOM in most clinical settings, however, it is expensive, and the availability is often limited or even absent. According to the diagnostic criteria as defined in the evidence-based guideline, the cut-off for PCOM is ≥ 20 follicles with a diameter of 2-9 mm in at least one ovary on transvaginal ultrasonography. As AMH strongly correlates with antral follicle count (AFC) on ultrasound, the 2023 guideline has now incorporated this as an alternative method to estimate PCOM. An AMH above the reference interval is regarded as PCOM.

5.1.2 Inclusion criteria

To be eligible for inclusion, each participant must meet each of the following criteria at Baseline (Visit 1):

- Signed informed consent and willingness to comply with the trial procedures
- Sufficient skills in the Finnish or Swedish language
- Participants with PCOS (diagnosed by the updated 2023 PCOS guideline)
- Participants 18-37 years of age at screening
- Participants with a BMI ≥ 25 kg/m2 and < 40 kg/m2

5.1.3 Exclusion criteria

Participants are excluded from the study if any of the following criteria is met at screening or at Baseline:

- Inability to provide written informed consent
- Participants not meeting the criteria by the updated PCOS guideline
- Participants with hormonal contraceptive during the last 3 months
- Participants that are pregnant or breastfeeding
- Participants with an untreated diabetes, hypothyroidism or hyperprolactinemia
- Use of medications for diabetes, high cholesterol, obesity and cortisone (per oral). Please see detailed list in Table 1.

- Participants with contraindication for use of metformin (hypersensitivity to metformin, acute metabolic acidosis, renal impairment, hepatic insufficiency, heart- or respiratory failure)
- Serious mental illness.
- Alcoholism
- Investigator site staff directly involved in the conduct of the study and their family members

5.1.4 Withdrawal of subjects from treatment

Participants who become pregnant during the study period or commence medication listed in Table 1, will be instructed to withdraw from the interventional component of a study, but we will still include them in the study and analyse blood samples and results from clinical examination and questionnaires, already performed.

Table 1 Instructions on inclusion and exclusion regarding medicine use in potential participants

Include	**Exclude**
Medication for hypothyroidism Medication for hyperprolactinemia Medication for mental illness (depression, anxiety) Local cortisone (lotion)	Metformin during the last 3 months Inositol Medication for diabetes Medication for hypercholesterolemia Medication for obesity Cortisone (per oral or iv) Hormonal contraceptive use during the last 3 months (including progesterone only substances) Medicines used for ovulation induction or IVF

Table 2 Selection criteria for inclusion and exclusion of studies in the RCT

	Include	**Exclude**
Population	Signed informed consent and willingness to comply with the trial procedures Participants with PCOS (by the updated 2023 PCOS guideline) Age: 18-37 years BMI: ≥ 25 kg/m2 and < 40 kg/m2 Subgroups: age, BMI, PCOS phenotype	-Participants not meeting the criteria of PCOS in the updated 2023 PCOS guideline (including a 17-OHP, TSH and PRL above reference) -Participants with hormonal contraceptive during the last 3 months -Participants that are pregnant or breastfeeding -Participants with an untreated diabetes, hypothyroidism or hyperprolactinemia

		-Use of medications for diabetes, high cholesterol, obesity and cortisone (per oral) -Participants with contraindication for use of metformin (hypersensitivity to metformin, acute metabolic acidosis, renal impairment, hepatic insufficiency, heart- or respiratory failure) -Serious mental illness. -Alcoholism -A planned surgical intervention during the next 26 weeks - Investigator site staff directly involved in the conduct of the study and their family members
Intervention	Metformin 1500mg/day for 26 weeks	NA
Comparison	Metformin 2250mg/day for 26 weeks	NA
Outcomes	**Anthropometric**: weight, height, BMI, WHR, BAI, body composition, BP, pulse, gastrointestinal effects, irregular cycles **Androgenicity**: Hirsutism, FAI, SHBG, DHEAS, testosterone, androstenedione, free testosterone **Metabolic**: HOMA-IR, fasting glucose, fasting insulin, HbA1C, lipids, ALAT, GGT, CRP, OGTT, FLI, Matsuda Index, TyG **Psychological**: Qol, depression, anxiety, weight stigma **Polycystic ovary morphology**: AMH, ultrasound (follicle number per ovary, ovarian volume)	NA

5.2 Randomisation and blinding

5.2.1 Randomisation

A double-blind, randomized controlled trial, including adults (age 18-37 years) with PCOS and a BMI ≥ 25 and <40 kg/m2 will be carried out. The randomization will be computer-generated and performed by the hospital pharmacy with 1:1 allocation in random blocks. We intend to do blinded analyses as well; hence the hospital pharmacy will provide the randomisation codes when the analyses have been performed.

5.2.2 Blinding

After the baseline visit has been performed, the participant will receive the medication, delivered by the University pharmacy to the Reproductive Medicine Unit (where all the visits will take place). The study will be conducted in a double-blind fashion. Thus, study treatment assignment will be blinded for both the investigators and the subject. The maintenance of clinical trial treatment randomisation codes will be done by the University pharmacy. The blinding can only be broken if a participant experiences serious adverse events (acute metabolic acidosis, renal impairment, hepatic insufficiency, heart- or respiratory failure). Any intentional or unintentional breaking of the blind will be recorded and reported to the sponsor as soon as possible.

5.3 Study assessments and procedures

Informed consent will be obtained prior to any study-related procedures being undertaken. The study is anticipated to last between 28 to 32 weeks after informed consent has been received. The schedule of assessments in the trial is summarised in Table 3.

Table 3. Schedule of events

Procedures	Call	Screen	Visit 1 Baseline	Visit 2 14 weeks	Visit 3 26 weeks
Day	D1 -2-4 weeks	D1 -0-2 weeks	D1	D1+14 weeks ±14 days	D1+26 weeks ±14 days
Eligibility Criteria	x	x			
Informed consent		x			
Medical history	x		x	x	x
Physical examination			x[a]	x[a]	x[a]
Vaginal ultrasound			x	x	x
Laboratory		x		x	x
Pregnancy test		x		x	x
Randomisation			x		
Dispensing of study medications			x	x	
Questionnaires (own and PCOSQ)		x		x	x
Adverse events				x	x
Medication compliance				x	x
Instructions on how to contact the research team if adverse events or questions	x	x	x	x	
Discussion on future treatment					x

[a]per investigator's discretion

5.3.1 Description of Study Assessments

Demographics: Age, BMI and ethnicity will be recorded.

Physical Examination: The physical examination will include height, weight, wc and WHR, BP, pulse, as well as hirsutism (mFG score)

Vaginal ultrasound: will include follicle number per ovary and ovarian volume

Laboratory tests: Please see Table 2 and Figure 1

Questionnaires: Two separate questionnaires are included in this study. The first one includes questions on medical history and adverse events, whereas the second one (PCOSQ) is a validated questionnaire developed specifically to measure quality of life for individuals with PCOS.

5.3.2 Endpoints assessments

Efficacy Assessment

Anthropometric:

- Weight and height: will be measured with participants allowed to wear light clothing after an overnight fasting. Weight will be measured by a standard scale to an accuracy of +/- 0,1kg. Height will be measured to an accuracy of +/- 0,1cm
- BMI (Body Mass Index): will be measured as kg/m^2
- WHR (Waist-hip-ratio). Will be calculated as waist measurement divided by hip measurement (W/H). Hip circumference is determined as the maximum value over the glottal region, measured to the nearest centimetre.
- WC (Waist circumference). Measured halfway between the participant's lowest rib and the top of the participant's hipbone
- BAI (Body adiposity index). Will be measured using the following equation: hip circumference (cm)/height (m) – 18
- BP (blood pressure) (systolic and diastolic) and pulse. Will be measured twice with at least 3 minutes apart after at least 10 min rest from non-dominant upper extremity in a subject in supine position

Metabolic:

All laboratory tests will be taken in the morning, between 7-10 am, after 12 hours fasting.

- HOMA-IR (Homeostatic model assessment for insulin resistance). Calculated with the formula: fasting plasma glucose (mmol/L) × fasting serum insulin (mU/L) divided by 22.5 (9)
- fasting glucose mmol/l (photometric hexokinase method)
- fasting insulin mU/l (immunochemiluminometric assay)
- HbA1C mmol/mol (photometric enzymatic method)
- Lipids (total cholesterol, mmol/l and triglycerides mmol/l with an enzymatic photometric method, LDL and HDL mmol/l with a direct enzymatic photometric method)
- CRP (C-reactive protein) mg/l (photometric immunochemical method)
- GGT U/l (photometric method according to IFCC recommendation)

- ALAT U/l (photometric method according to IFCC recommendation)
- 2-hour OGTT mmol/l
-
- From these tests, following indexes can be calculated: FLI, Matsuda Index, TyG index

Androgenicity:

Clinical:

- Hirsutism. Modified Ferriman Gallwey score (mFG) will be measured and a score of 4 or more will be regarded as hirsutism in Caucasian women, whereas a score of 6 or more will be regarded hirsutism for other ethnicities.

Biochemical:

All laboratory tests will be taken in the morning, between 7-10 am, after 12 hours fasting.

- Testosterone nmol/l (liquid chromatography with tandem mass spectrometry (LC-MS/MS))
- FAI (free androgen index) (FAI = 100 X ((Total Testosterone (nmol/L)/SHBG (nmol/L))
- SHBG (sex hormone binding globulin) nmol/l (immunochemiluminometric assay)
- DHEAS (dehydroepiandrosterone sulfate) nmol/l (immunochemiluminometric assay)
- Androstenedione nmol/l (liquid chromatography with tandem mass spectrometry (LC-MS/MS))
- free calculated testosterone pmol/l (by Anderson Formula) (8).

Menstrual Cyclicity:

- Questionnaire, where asking if the menstrual cycle is regular (defined as 21 days or more but under 36 days)
- Questionnaire, where asking how long the two previous cycles were (days)

Side-effects:

- Questionnaire, where asking about the appearance and severeness of the following symptoms: abdominal pain, reflux, nausea, diarrhoea, loss of appetite**.**

Quality of life and weight stigma:

- The PCOS-Q- a validated questionnaire that was specifically designed to measure quality of life in women with PCOS.

Polycystic ovary morphology:

- AMH (anti-Mullerian hormone) (ug/l) will be taken in the morning, between 7-10 am, after 12 hours fasting (with other laboratory measurements).
- Vaginal ultrasound where we measure follicle number per ovary and ovarian volume will be measured at any stage of the cycle.

The primary timepoint for this study is 26 weeks, whereas the 14 weeks timepoint will be secondary.

Safety Assessment

Safety evaluations will be performed as adverse event monitoring during the 14 week and 26 week visits. In case of acute symptoms of acute metabolic acidosis, renal impairment, hepatic insufficiency, heart- or respiratory failure, patients will be asked to call the emergency department. Participants will be instructed to do a home pregnancy test during week 4, 8, 12, 18 and 22 of the trial. If the participant becomes pregnant, we will instruct them to withdraw from the interventional component of the study (discontinue the metformin-medication), but we will still include them in the study and analyse blood samples and results from clinical examination and questionnaires, already performed.

For further information on safety assessments, please see the summary of product characteristics.

5.4 Study visit structure

5.4.1 Screening procedure

Screening call:

1. Potential participants for the study will be recruited from individuals referred to the Reproductive Medicine Unit at Helsinki University Hospital in Finland and by advertising via the Finnish patient organization for those with PCOS (Korento). Potential participants will be sent an electronic link to express interest in participating in the study
2. Check the age (18-37 years) and BMI (BMI≥ 25 kg/m2 and <40 kg/m2) of the individuals that have expressed interest in participating in the study
3. Ask the individual on signs of PCOS (menstrual irregularities, biochemical and clinical hyperandrogenism, PCOM)
4. Ask if the individual is pregnant or breastfeeding, illnesses and medication use
5. Exclude the participants that don't meet the inclusion criteria
6. Describe the study in detail
7. If the participant is eligible and willing to participate in the study, send written information and letter for informed consent to home address
8. Book a screening visit within 2 weeks

Screening visit:

1. Collect the signed informed consent
2. Check the age (18-37 years) and BMI (BM I≥ 25 and <40 kg/m2) of the woman. Weight and height measurement if needed!
3. Check the inclusion and exclusion criteria
4. Answer possible questions that the participant might have
5. Refer the participant to laboratory and give her the questionnaires
6. Book a baseline visit within 2 weeks from the screening visit

Individuals who are incapable of giving informed consent will be excluded from the study and informed consent will be obtained prior to any study related procedures being undertaken.

The maximum duration allowed between screening call and randomisation is 28 days and 14 days between screening visit and randomisation.

Date of screening, age of the individual and reason for ineligibility (if the individual is not eligible) will be recorded. The results of the screening evaluation must meet the inclusion/exclusion criteria for the individual to continue in the study.

Laboratory check:

1. Check the laboratory results and make sure the participant is eligible for the study

5.4.2 Baseline assessments (+0-14 days, Visit 1)

1. Clinical examination (hirsutism – mFG score, weight, height whr, BP)
2. Vaginal ultrasound (FNPO, ovarian volume)
3. Collection of filled questionnaires (own and PCOSQ)
4. Inform the participants how metformin is taken (1 tabl per day for the first week, 2 tabl per day for the second week, 3 tabl per day starting third week and always together with a meal).
5. Ask if the participants wish to receive condoms as contraception and provide if needed
6. Give the participant metformin for the next 14 weeks (delivery of medication will be made by the university pharmacy)
7. Instruct the participant to do home pregnancy test on week 4,8 and 12 and contact us for exclusion from the study and termination of the medication if pregnant
8. Ask the participant to bring with her the Metformin medication not used
9. Refer for laboratory tests and give the link to questionnaires to be filled out for next appointment
10. Book the next appointment

5.4.3 Visit 2 (+14 weeks +/-14 days)

1. Check the laboratory results and make sure the participant can continue the study (not pregnant, no serious, adverse events)
2. Check and document how much metformin is unused
3. Clinical examination (hirsutism – mFG score, weight, whr, BP)
4. Vaginal ultrasound (FNPO, ovarian volume)
5. Collection of filled questionnaires (own and PCOSQ)
6. Ask if the participants wish to receive condoms as contraception and provide if needed
7. Give the participant metformin for the next 12 weeks (delivery of medication will be made by the university pharmacy)
8. Instruct the participant to do home pregnancy test on week 18 and 22 and contact us for exclusion from the study and termination of the medication if pregnant
9. Ask the participant to bring with her the metformin medication not used
10. Refer for laboratory tests and give the link to questionnaires to be filled out for next appointment

11. Provide a card with sponsor contact details in case emergency unblinding becomes necessary.
12. Book the next appointment

5.4.4 End-of-Treatment visit (+12 weeks +/- 14 days, Visit 3)

1. Check the laboratory results
2. Check and document how much metformin is unused
3. Clinical examination (hirsutism – mFG score, weight, whr, BP)
4. Vaginal ultrasound (FNPO, ovarian volume)
5. Collection of filled questionnaires (own and PCOSQ)
6. Discussion on future treatment and follow up (continuation of metformin etc)

5.5 End-of-trial

5.5.1 Definition

The clinical trial will end after the last participant has attended the last visit (Visit 3). If one of the participants in the trial falls pregnant, the clinical trial will end at the latest 38 weeks after Visit 3 of the last pregnant participant. For pregnant participants, we will search the hospital charts to evaluate the endpoint of the pregnancy (miscarriage, extrauterine pregnancy, induced abortion or live birth).

The Sponsors and/or the trial steering committee have the right at any time to terminate the study for clinical or administrative reasons.

5.5.2 Premature termination of the study

The study or a part of the study may be stopped if new information about safety or efficacy is received.

If a participant becomes pregnant, starts a medication on "the exclusion list" (Table 1) or gets sever adverse events (acute metabolic acidosis, renal impairment, hepatic insufficiency, heart- or respiratory failure), they will be instructed to withdraw from the interventional component of the study (discontinue the metformin medication), but we will still include them in the study and analyse blood samples and results from clinical examination and questionnaires, already performed.

Participants have the right to voluntarily discontinue study treatment or withdraw from the study at any time for any reason without any consequences.

5.5.3 Reporting the end of trial

The end of the study will be reported in the CTIS (Clinical Trials Information System) within 15 days from the end of the clinical trial.

5.6 Discontinuation/withdrawal of subjects from study protocol

Participants have the right to voluntarily discontinue study treatment or withdraw from the study at any time for any reason without any consequences. The participant is not obliged to disclose the reason for discontinuation. The investigator has the right to discontinue a subject from study treatment or withdraw a subject from the study at any time if it is in the best interest of the subject.

Subjects can be withdrawn from the study or the interventional component for any of the following reasons:

- withdrawal of consent by the subject
- any medical condition that the investigator or sponsor determines may jeopardize the subject's safety if she or he continues receiving the study treatment
- pregnancy
- ineligibility (either arising during the study or retrospectively having been overlooked at screening)
- an adverse event which requires discontinuation of the study medication
- lack of compliance with the study or study procedures (e.g., dosing, study visits)
- lost to follow-up (at least three documented attempts must be made to contact any subject lost to follow-up)

If a subject is withdrawn before completing the study, the reason for withdrawal will be entered on the appropriate case report form (CRF) page.

If a subject is withdrawn due to an adverse event, the investigator will arrange for follow-up visits until the adverse event has resolved or stabilise

If a subject is withdrawn before visit 2 (less than 14 weeks metformin use), she will be replaced by another subject. If she is withdrawn later, she will not be replaced. We will use intention-to treat-analyses in this study.

5.7 Participant care after the trial

During the last visit, we will inform the participant on the recommended follow up for individuals with PCOS, which is generally clinical examination and laboratory tests every 1-3 years. If the participant is willing to continue and fulfils the criteria for metformin medication, a prescription will be written. The follow-up should be arranged at the participants health care centre or in private care by the participant herself.

6 TREATMENT OF TRIAL SUBJECTS

6.1 Description of study treatment(s)

Participants in the study will be divided into two arms, receiving a different dose of metformin:
1. Metforem depotablet (extended release) 500 mg, containing 500 mg of metformin hydrochloride, used at a maximum dose of one tablet in the morning and two in the evening.
Size: 19 x 8,8 mm

Colour: White

2. Metforem depotablet (extended release) 750 mg, containing 750 mg of metformin hydrochloride, used at a maximum dose of one tablet in the morning and two in the evening

Size: 20,3 x 9,4 mm

Colour: White

Metformin is a low cost, readily available medication that has been extensively used as an insulin sensitiser for over seven decades in type 2 diabetes mellitus and for several decades in PCOS.

The optimal metformin dose for adults with PCOS is unclear but the suggested maximum daily dose according to the International PCOS Guideline is 2.5 g in adults (6). Metformin should be taken together with a meal.

6.2 Formulation, packaging and handling

The packing and labelling of the metformin medication will be done by Meilahti University Pharmacy (address: Siltasairaalan aula, Haartmaninkatu 4, 00290 Helsinki). We are currently negotiating with the pharmaceutical company Orion Pharma, whether they would be willing to donate metformin medication for the study.

6.3 Storage and disposition of study treatment(s)

The metformin medication will be stored according to instructions from the pharmaceutical company at the Meilahti University Pharmacy. We anticipate doing research visits once per week and the metformin medication will be brought from Meilahti University Pharmacy earlier at the same day of the research visits. The metformin medication will be stored and locked in a secure place until they are dispensed for subject use or are returned to the sponsor. There are no specific instructions for storage conditions of metformin medication by the pharmaceutical company. The metformin medication is for investigational use only and is to be used only within the context of this study.

6.4 Accountability of the study treatment(s)

The investigator is responsible for the control of the metformin medication during the investigation. A table of distributed and returned number of metformin tablets, with dates and package identification number, per each subject will be used for documenting the accountability.

The study medication will be supplied to Meilahti University Pharmacy by Orion Pharma and retrieved at the end of the study.

The investigator will use a standard prescription manner of the institution, and the investigator will collect the medication from the pharmacy.

6.5 Assessment of compliance

The participants will be asked to bring all unused metformin tablets to their next visit. Compliance will be assessed by maintaining dispensing records.

If a participant has used less than an average of 2,5 metformin tablets per day (after expected to use 3 tablets per day), it will be regarded as significant non-compliance.

When significant non-compliance is observed, the reason behind non-compliance will be evaluated (forgetting medication, side-effects etc). Efforts to find ways to minimize non-compliance will be taken (alarm if forgetting medication, timing of medicine intake if side-effects etc).

Participants will also be encouraged to contact the research team if they are bothered by or experiences severe side effects or have difficulties with using the medication as instructed.

6.6 Overdose of study treatment

If overdose of metformin is observed or comes to the knowledge of the research team, the situation will be handled according to the dose of metformin. According to information received from the national Poison Center (Myrkytystietokeskus), if a suspected dose of over 6000mg per day has been used (8 tablets or more), the participant will be asked to contact the emergency

unit. If a dose of more than 3000mg (4 tablets or more) has been used and the participant experiences serious side effects (signs of acute metabolic acidosis, renal impairment, hepatic insufficiency, heart- or respiratory failure) the participant will be asked to contact the emergency unit.

6.7 Prior and concomitant treatment

Any non-study medication during the trial will be recorded during the study period. Medication use will be documented at the screening call, screening visit, visit 1, 2, and 3.

6.7.1 Permitted medications

Permitted medications are described in Table 1

6.7.2 Prohibited medications

Prohibited medications are described in Table 1. If a prohibited medication is commenced during the study, the participant will be excluded from the interventional component of the study, starting from the intake of the prohibited medication.

7 SAFETY REPORTING

The safety and tolerability will be evaluated throughout the study based on adverse events, vital signs and physical exam findings. The appearance of adverse events will be asked in a questionnaire and documented on visit 2 and 3. In addition, the participants will be instructed to contact the research team (by phone or email) if adverse events occur during the study period.

7.1 Definitions

7.1.1 Adverse event

Adverse events *are* any untoward medical occurrence in a patient or clinical trial participant administered a medicinal product, which do not necessarily have a causal relationship with this treatment.

An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of a medicinal product, even if not considered related to the medicinal product.

7.1.2 Adverse reaction

All untoward and unintended responses to a medicinal product related to any dose count as adverse reactions. The phrase 'responses to a medicinal product' means that a causal relationship between a study medication and an adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the sponsor as having a reasonable suspected causal relationship to the study medication qualify as adverse reactions.

7.1.3 Serious adverse event (SAE)

Any untoward medical occurrence or affect that at any dose:

- 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 or birth defect
- counts as an important medical event**

*Regarding a life-threatening event, this refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

**Some medical events may jeopardise the subject or may require an intervention to prevent one of the above characteristics/consequences. Such events (hereinafter referred to as 'important medical events') should also be considered as 'serious' in accordance with the definition

7.1.4 Suspected unexpected serious adverse reactions (SUSARs)

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. undesirable events listed in the SmPC section 4.8)

7.2 Evaluation of adverse events and SAEs

7.2.1 Assessment of seriousness

In previous studies the reported adverse effects with metformin are usually mild and self-limiting including nausea, vomiting, diarrhoea and abdominal pain. Lactic acidosis, a previously feared serious adverse effect, is very rare in women with PCOS, 1 in 30,000 patients using metformin (9). Adverse effects can be minimized by starting metformin treatment at a lower dose with 500mg increments 1-2 times weekly and by using extended-release preparations (3, 10).

7.2.2 Assessment of causality

All adverse events judged by either the investigator or the sponsor as having a reasonable suspected causal relationship to an investigational medicinal product qualify as adverse reactions.

All adverse events judged as being related to an interaction between the study medication and another medication will also be considered adverse reactions.

Alternative causes such as natural history of the underlying disease, concomitant therapy, other risk factors and the temporal relationship of the event to the treatment should be considered.

7.2.3 Assessment of intensity

The investigator will assess severity for each adverse event and record this on the Case report form (CRF) according to one of the following categories:

Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities (grade 1-3 in the questionnaire)

Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities (grade 4 in the questionnaire)

Severe: An event that prevents normal everyday activities (grade 5 in the questionnaire)

7.2.4 Assessment of expectedness

Adverse gastrointestinal effects, such as diarrhoea, nausea, loss of appetite and vomiting, are relatively common, affecting up to 30% of patients who take metformin. Lactic acidosis is a rare, yet severe adverse effect with an incidence rate of approximately 1 in 30,000 patients using metformin. Several risk factors contribute to developing lactic acidosis, including hepatic or renal impairment, advanced age and alcoholism. Participants with these risk factors will be excluded from the study. Overall Metformin is generally considered safe and well-tolerated. For

more details, please see the SmPC section 4.8., which will be considered as reference safety information (RSI) for this trial for the assessment of expectedness.

7.2.5 Emergency unblinding procedures

In case of a serious adverse event or overdosing, where the participant will receive emergency medical care, the study blind will be broken. This can be done by contacting the research team by phone, who will make the decision and perform the unblinding. Participants will be provided with and encouraged to carry a card with sponsor contact details in case emergency unblinding becomes necessary.

7.3 Reporting procedures for all adverse events

All adverse events occurring during the study observed by the investigator or reported by the subject will be recorded on the CRF.

Serious adverse events considered related to the study medication as judged by an investigator will be followed until resolution or until the event is considered stable. All related serious adverse events that result in a subject's withdrawal from the interventional component of the study or are present at the end of the study, will be followed up until a satisfactory resolution occurs.

It will be left to the investigator's clinical judgment whether an adverse event is of sufficient severity to require the subject's removal from treatment. A subject may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable adverse event. If either of these occurs, the subject will undergo an end-of-study assessment and be given appropriate care under medical supervision until symptoms cease or the condition becomes stable.

Any pregnancy occurring during the clinical study and the outcome (e.g., delivery of healthy child at term, spontaneous abortion) of the pregnancy will be recorded and followed.

7.4 Reporting procedures for serious adverse events (SAE)

The investigator will report all SAE immediately to the sponsor. The immediate report will be followed by detailed, written reports. The immediate and follow-up reports will identify subjects by unique code numbers assigned to the latter. The immediate report will be made by the investigator to the sponsor within 24 h following knowledge of the serious adverse event.

All SAE information must be recorded on an SAE form and sent expeditiously to the sponsor. Additional information received for a case (follow-up or corrections to the original case) need to be detailed on a new SAE form and sent expeditiously to the sponsor.

The sponsor will keep detailed records of all adverse events reported by the investigator or investigators.

In cases where reporting is not required immediately the investigator will report within the appropriate time frame, taking account of the specificities of the trial and of the serious adverse event, as well as possible guidance in the protocol or the investigator's brochure.

The academic sponsors will report all SUSARs to FIMEA, using CIOMS-I template. Fatal or life-threatening SUSARs must be reported within 7 days. SUSARs which are not fatal and not life-threatening are to be reported within 15 days. The sponsor will also inform all investigators

concerned of relevant information about SUSARs that could adversely affect the safety of subjects.

If the initial report is incomplete, e.g. if the sponsor has not provided all the information within seven days, the sponsor will submit a completed report based on the initial information within an additional eight days.

If significant new information on an already reported case is received by the sponsor, the clock starts again at day zero, i.e. the date of receipt of new information. This information will be reported as a follow-up report within 15 days.

In addition to the expedited reporting above, the sponsor will, once a year throughout the clinical trial or on request, submit a safety report to the competent authority and ethics committees.

7.5 Pregnancy

The investigators will collect pregnancy information on all participants. All pregnancies during the trial will be followed to outcome.

8 STATISTICS

8.1 Description of statistical methods

Continuous variables will be summarised by number of patients, mean, standard deviation, median, minimum, and maximum values and by reporting mean differences. Categorical variables will be summarized by counts and percentages.

Primary analysis: For normally distributed variables, independent-sample t-tests will be used to evaluate changes between measurements at baseline and after 14 and 26 weeks of treatment and to compare the different metformin doses. Mann-Whitney will be performed for variables with skewed distribution.

Supplementary analysis: We will also use repeated measures ANOVA and linear mixed-effects models when comparing the two metformin doses regarding change from baseline measurement and when comparing the two groups to each other.

Adverse event rates between the two treatment arms will be calculated using Poisson regression models. We will follow the intention-to-treat principle and adjust/perform sub-analyses according to age, BMI and PCOS phenotype.

8.2 Determination of sample size subjects

As we have found only one previous RCT comparing different metformin doses in PCOS (11) where standard deviations (SD) were not calculated, it is impossible to use previous RCT studies on different metformin doses to base decisions on SD and margin on.

In the research group we have agreed on 6 primary endpoints with the following standard deviations and non-inferiority limits. For non-inferiority analyses we have used a power calculator found on www.sealedenvelope.com/power/continuous-noninferior/

For superiority analyses we have used G Power 3.1.

Weight: For this outcome we have used a non-inferiority test. In the study by Harborne et al. (11), a weight difference of 2.1 kg was non-significant. Generally, a weight reduction of 5%, being 4 kg for a person with the weight of 80 kg, is regarded as a significant weight reduction (12), which is why the research team decided to use 4 kg as a margin. Power analysis has indicated that a total number of 156 participants (78 in each group) will be needed to reveal a 4kg weight reduction difference between the groups, with an 80% power. Here we have used a non-inferiority limit of 4kg, a SD of 10 and a 5% p-value. We have estimated a 15% drop-out making the total number of participants needed to **184 (92 participants in each group).**

HOMA-IR (Homeostatic model assessment for insulin resistance): For this outcome we have used a non-inferiority test. We have not found any studies on what is considered a significant HOMA-IR reduction. Based on an expert opinion by endocrinologist and adjunct professor Heikki Koistinen we decided on a reduction of 20%. According to a recent study (13), the cut-off value for HOMA-IR to discriminate insulin resistance in women with PCOS is 2.1 and based on this we have decided on a margin of 0.42. Power analysis has indicated that a total number of 142 participants (71 in each group) will be needed to reveal a 0.5 reduction difference of HOMA-IR between the groups, with an 80% power. Here we have used a non-inferiority limit of 0.42, a SD of 1.0 and a 5% p-value. We have estimated a 15% drop-out, making the total number of participants needed to **168 (84 participants in each group).**

Hirsutism: For this outcome we have used a non-inferiority test. According to a study by Harborne et al. (12), a reduction of 2 mFG-score was significant when studying the effect of metformin on hirsutism, which is why the research team decided to use this margin. Power analysis has indicated that a total number of 156 participants (78 in each group) will be needed to reveal a reduction difference of 2 mFG scores between the groups, with an 80% power. Here we have used a non-inferiority limit of 2, a SD of 5 and a 5% p-value. We have estimated a 15% drop-out, making the total number of participants needed to **184 (92 participants in each group).**

Testosterone: For this outcome we have used a non-inferiority test. In a study comparing different metformin doses (1000mg and 1500mg) in breast cancer patients (14), a testosterone decrease of 0.41 was considered significant, which is why the research team decided to use this margin. Power analysis has indicated that a total number of 148 participants (74 in each group) will be needed to reveal a 0.41 reduction difference of Testosterone between the groups, with an 80% power. Here we have used a non-inferiority limit of 0.41, a SD of 1.0 and a 5% p-value. We have estimated a 15% drop-out, making the total number of participants needed to **174 (87 participants in each group).**

AMH (anti-Mullerian hormone): For this outcome we have used a non-inferiority test. Several factors affect AMH in the general population, including laboratory assays, age, BMI, ethnicity, menstrual cycle stage and ovarian surgery (15). A recent study has showed that for the Elecsys assay (used by HUSLAB) AMH varies 0.7-0.31 pmol/l per year according to age (17). The intercycle variation is expected to be small (16). In our research team, we decided on a reduction of 0.5 pmol/l. Power analysis has indicated that a total number of 100 participants (50 in each group) will be needed to reveal a 0.5 reduction difference of AMH between the groups, with an 80% power. Here we have used a non-inferiority limit of 0.5, a SD of 1.0 and a 5% p-value. We have estimated a 15% drop-out, making the total number of participants needed to **118 (59 participants in each group).**

Gastrointestinal side-effects: Gastrointestinal adverse events of metformin are the most common and are assessed to affect up to 20% of patients (18). For this outcome we have used a superiority test (Fisher's exact test). Power analysis has indicated that a total number of 138 participants (69 in each group) will be needed to reveal a 20% difference in side-effect between the groups, with an 80% power. Here we have used a two tailed test with proportions of 0,30 and 0,10 and a 5% p-value. We have estimated a 15% drop-out, making the total number of participants needed to **164 (82 participants in each group).**

The publication on quality of life/mental health and weight stigma in women with PCOS is more of exploratory than confirmatory nature, which is why we have not defined a primary outcome for this publication.

In this study we will have 1-2 primary outcome per publication, the rest will be secondary outcomes of more exploratory nature. Hence, we have not planned to perform multiplicity adjustments in our analyses. For the publication on hyperandrogenism, where two primary outcomes (hirsutism and total testosterone) are listed, hirsutism as a marker of clinical hyperandrogenism will be of higher hierarchy.

Our hypothesis is that individuals using the lower metformin dose (1500 mg/day) might improve their biochemical and clinical outcomes to the same extent than those using a higher metformin dose (2250 mg/day) but with fewer gastrointestinal side effects.

8.3 Analysis sets

The set of participants whose data are to be included in the analyses are defined in the statistical section of the protocol, 8.1 and 8.2

8.4 Demographic and baseline disease characteristics

Demographic and baseline disease characteristics will be summarised descriptively by treatment group.

8.5 Efficacy analysis

8.5.1 Primary and secondary efficacy endpoint

Anthropometric:

Primary outcome: Weight
Secondary outcomes: BMI, WC, WHR, BAI, body composition, BP, pulse

Here we will measure change in outcomes from baseline to 14 and 26 weeks. We will summarise the outcomes by number of patients, mean, standard deviation, median, minimum, and maximum values and report mean differences. We will follow the intention-to-treat principle and adjust/perform sub-analyses according to age, BMI and PCOS phenotype.

Primary analysis: For normally distributed variables, independent-sample t-tests will be used to compare values between and within the two treatment groups at baseline and 26 weeks. Mann-Whitney will be performed for variables with skewed distribution.

Supplementary analysis: Repeated measures ANOVA will be performed to estimate the within-group modifications (baseline, 14 and 26 weeks). We will also use linear mixed-effects models when comparing the two metformin doses regarding change from baseline measurement and when comparing the two groups to each other.

Metabolic:

Primary outcome: HOMA-IR

Secondary outcomes: fasting glucose, fasting insulin, HbA1C, lipids (cholesterol and its subclasses, triglycerides), CRP, 2-hour OGTT, ALAT, G-GT, FLI, Matsuda Index, TyG index

For all outcomes we will measure change from baseline to 14 and 26 weeks. We will summarise the outcomes by number of patients, mean, standard deviation, median, minimum, and maximum values and report mean differences. We will follow the intention-to-treat principle and adjust/perform sub-analyses according to age, BMI and PCOS phenotype.

Primary analysis: For normally distributed variables, independent-sample t-tests will be used to compare values between and within the two treatment groups at baseline and 26 weeks. Mann-Whitney will be performed for variables with skewed distribution.

Supplementary analysis: Repeated measures ANOVA will be performed to estimate the within-group modifications (baseline, 14 and 26 weeks). We will also use linear mixed-effects models when comparing the two metformin doses regarding change from baseline measurement and when comparing the two groups to each other.

Androgenicity:

Clinical:

Primary outcome: hirsutism

Secondary outcome: acne, alopecia

For hirsutism, we will measure modified Ferriman-Gallwey score as a part of the clinical examination and for acne we will use the PCOS-Q to ask about facial hirsutism and alopecia (score 1-7). We will report change from baseline to 14 and 26 weeks by number of patients, mean, standard deviation, median, minimum, and maximum values and report mean differences. We will follow the intention-to-treat principle and adjust/perform sub-analyses according to age, BMI and PCOS phenotype.

Primary analysis: For normally distributed variables, independent-sample t-tests will be used to compare values between and within the two treatment groups at baseline and 26 weeks. Mann-Whitney will be performed for variables with skewed distribution.

Supplementary analysis: Repeated measures ANOVA will be performed to estimate the within-group modifications (baseline, 14 and 26 weeks). We will also use linear mixed-effects models when comparing the two metformin doses regarding change from baseline measurement and when comparing the two groups to each other.

Biochemical:

Primary outcome: total testosterone
Secondary outcomes: FAI, SHBG, DHEAS, androstenedione, free testosterone

For all outcomes we will measure change from baseline to 14 and 26 weeks. We will summarise the outcomes by number of patients, mean, standard deviation, median, minimum, and maximum values and report mean differences. We will follow the intention-to-treat principle and adjust/perform sub-analyses according to age, BMI and PCOS phenotype.

Primary analysis: For normally distributed variables, independent-sample t-tests will be used to compare values between and within the two treatment groups at baseline and 26 weeks. Mann-Whitney will be performed for variables with skewed distribution.

Supplementary analysis: Repeated measures ANOVA will be performed to estimate the within-group modifications (baseline, 14 and 26 weeks). We will also use linear mixed-effects models when comparing the two metformin doses regarding change from baseline measurement and when comparing the two groups to each other.

Menstrual Cyclicity:

Secondary outcomes: questionnaire, where asking if the menstrual cycle is regular (defined as 21 days or more but under 36 days) and how long the previous two cycles were (days).

Continuous variables (cycle length) will be summarised by number of patients, mean, standard deviation, median, minimum, and maximum values and by reporting mean differences. Categorical variables (regular cycle – yes/no) will be summarized by counts and percentages.

We will follow the intention-to-treat principle and adjust/perform sub-analyses according to age, BMI and PCOS phenotype.

Primary analysis: Rates of regular menstrual cycle between the two treatment arms will be calculated using Poisson regression models. For normally distributed variables, independent-sample t-tests will be used to compare continuous variables between and within the two treatment groups at baseline and 26 weeks. Mann-Whitney will be performed for variables with skewed distribution.

Supplementary analysis: Repeated measures ANOVA will be performed to estimate the within-group modifications (baseline, 14 and 26 weeks). We will also use linear mixed-effects models when comparing the two metformin doses regarding change from baseline measurement and when comparing the two groups to each other.

Gastrointestinal side-effects:

Primary outcome: Appearance of severe of adverse effects.

Secondary outcomes: Appearance of GI AE (never/everything else but never), subanalyses on the appearance of different adverse effects (abdominal pain, reflux, nausea, diarrhoea, loss of appetite) and discontinuation of metformin due to GI AE.

In our questionnaire we will ask the participants whether they have experienced abdominal pain, reflux, nausea, diarrhoea or loss of appetite during the last two weeks and grade the symptoms from 1-5 (1 being never and 5 being all the time). We will count the scores and give each participant a score between 5-25. A severe adverse effect is defined as a score of 4 or more in one of the questions (indicating a daily experience of abdominal pain, reflux, nausea, diarrhoea or loss of appetite during the last two weeks). We will measure change from baseline to 14 and 26 weeks and summarise the outcomes by number of patients, mean, standard deviation, median, minimum, and maximum values and report mean differences. We will follow the intention-to-treat principle and adjust/perform sub-analyses according to age, BMI and PCOS phenotype.

Primary analysis: Adverse event rates between the two treatment arms will be calculated using Poisson regression models. For normally distributed variables, independent-sample t-tests will be used to compare values between and within the two treatment groups at baseline and 26 weeks. Mann-Whitney will be performed for variables with skewed distribution.

Supplementary analysis: Repeated measures ANOVA will be performed to estimate the within-group modifications (baseline, 14 and 26 weeks). We will also use linear mixed-effects models when comparing the two metformin doses regarding change from baseline measurement and when comparing the two groups to each other.

Quality of life and weight stigma:

Secondary outcomes: The PCOS-Q is a questionnaire that was specifically designed to measure quality of life for women with PCOS. Questions on depression, anxiety, fear etc will be given scores from 1-7, depending on severity. In our own questionnaire we will ask about weight stigma and ask for examples on it. This publication will be of more exploratory nature. We will, however, count the scores and measure change from baseline to 14 and 26 weeks and summarise the outcomes by number of patients, mean, standard deviation, median, minimum, and maximum values and report mean differences.

Primary analysis: Rates between the two treatment arms will be calculated using Poisson regression models. For normally distributed continuous variables, independent-sample t-tests will be used to compare values between and within the two treatment groups at baseline and 26 weeks. Mann-Whitney will be performed for variables with skewed distribution.

Supplementary analysis: Repeated measures ANOVA will be performed to estimate the within-group modifications (baseline, 14 and 26 weeks). We will also use linear mixed-effects models when comparing the two metformin doses regarding change from baseline measurement and when comparing the two groups to each other.

Polycystic ovary morphology:

Primary outcome: AMH

Secondary outcomes: Follicle number per ovary, antral follicle count (AFC) and ovarian volume

For all outcomes we will measure change from baseline to 14 and 26 weeks. We will summarise the outcomes by number of patients, mean, standard deviation, median, minimum, and maximum values and report mean differences.

Primary analysis: For normally distributed variables, independent-sample t-tests will be used to compare values between and within the two treatment groups at baseline and 26 weeks. Mann-Whitney will be performed for variables with skewed distribution.

Supplementary analysis: Repeated measures ANOVA will be performed to estimate the within-group modifications (baseline, 14 and 26 weeks). We will also use linear mixed-effects models when comparing the two metformin doses regarding change from baseline measurement and when comparing the two groups to each other. We will follow the intention-to-treat principle and adjust/perform sub-analyses according to age, BMI and PCOS phenotype.

8.6 Safety analysis

The appearance of adverse events is asked in the questionnaire on visit 2 and 3 and grouped into five different categories (see questionnaires attached).

8.7 The level of statistical significance

The level of statistical significance to be used in this study is <0,05

8.8 Criteria for the termination of the trial

The trial will be terminated when at least 92 participants per treatment arm have attended visit 2.

Since Metformin is generally considered a safe and well-tolerated medication, the risk for having to terminate the trial due to too many serious adverse events is small.

8.9 Procedure for accounting for missing, unused and spurious data

We will perform intention-to-treat analyses. To reduce the number of participants without any treatment data, we will measure outcomes at 14 weeks to have some data available if the participant falls pregnant or decides to terminate the study.

8.10 Procedure for reporting any deviation(s) from the original statistical plan

Any deviations from the original statistical plan will be discussed with the other research members.

9 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Direct access to study documents and source data will be granted to authorised representatives from the sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

10 DATA HANDLING AND RECORD KEEPING

10.1 Data collection, source documents and case report forms

Source documents for this study will include hospital records, procedure reports and data collection forms. These documents will be used to enter data on the CRFs. All data entered on CRFs will be entered legibly. If an error is made, the error will be crossed through with a single line in such a way that the original entry can still be read. The correct entry will then be clearly inserted, and the alterations will be initialled and dated by the investigator.

Data reported on the CRF that are derived from source documents must be consistent with the source documents or the discrepancies must be explained.

All documents will be stored safely in confidential conditions. On all study-specific documents other than the signed consent, the subject will be referred to by the study subject identification code.

10.2 Data reporting

All study visits will be documented in the participant's hospital records. Information on QoL and adverse effects will be documented in the questionnaire, filled by the participant. There will be source data on all data recorded on the CRF. On all study-specific documents other than the signed consent, the subject will be referred to by the study subject identification code.

11 RETENTION OF ESSENTIAL DOCUMENTS

Essential documents will be retained for at least 25 years from study end. We will retain the trial-related essential documents as required by the applicable regulatory requirements and until the sponsor informs us or the institution that these documents are no longer necessary.

12 QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

To ensure that the data obtained from this research are accurate, complete, and reliable, data entered on the CRF will be independently double checked by two investigators.

13 AUDITS AND INSPECTIONS

This trial may be subject to internal or external auditing or inspections procedure to ensure adherence to GCP. Access to all trial-related documents will be given at that time.

14 ETHICAL CONSIDERATIONS

Metformin, especially in the early stages of treatment, can cause gastrointestinal side effects, such as diarrhea, nausea and loss of appetite. Most of the time, the side effects are mild and self-limiting and disappear as the treatment continues.

In our opinion, this study meets the regulations and principles set for medical research and the safety of the subjects has been considered. We consider the research to be ethical.

14.1 Declaration of Helsinki

The sponsor will ensure that this study is conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki.

14.2 Good Clinical Practice

This study will be conducted in accordance with Good Clinical Practice, as defined by the International Conference on Harmonisation and set out in directive 2005/28/EC as well as in accordance with the ethical principles underlying European Union Regulation (EU) No 536/2014.

14.3 Approvals

Required documents including the protocol, informed consent form, subject information leaflet, investigational medicinal product dossier, investigators brochure and any other required documents will be submitted to the ethical committee at Helsinki University for written approval.

The sponsor will submit and obtain approval from the above parties for substantial modifications to the original approved documents.

14.4 Informed consent

The investigators will take informed consent and explain the nature of the study to the subject and answer all questions regarding this study. Prior to any study-related screening procedures being performed on the subject, the informed consent statement will be reviewed and signed and dated by the subject and the investigator who administered the informed consent form.

A representative subject information leaflet and a sample subject consent form is attached as a separate document.

14.5 Benefits and risks assessment

The benefits of this study for the participants with PCOS is that they will receive a thorough medical examination, information and treatment for their condition. The risks and burden associated with participation include gastrointestinal side effects from the medication and minor pain related to the blood samples. The risks connected to this study can be considered minimal.

14.6 Subject confidentiality

The trial staff will ensure that the subjects' anonymity is maintained. The subjects will be identified only by initials and a subject's identification number on the CRF and any database. All documents will be stored securely. The study will comply with the EU General Data Protection Regulation 2016/679.

The sponsor shall be notified immediately upon a data security breach observed. The sponsor will promptly analyse the case and notify regulatory bodies as indicated.

14.7 Retention of biological samples

Please see the separate document "compliance_with_use_biological_samp_en"

15 FINANCING AND INSURANCE

Helsinki University holds an insurance for the participants.

At the moment two grants have been received:

1. Valtion tutkimusraha (VTR) - 65,311€
2. Finska Läkaresällskapet – 11,250€

Altogether: 76561€ for the first year

We are also negotiating with pharmaceutical companies to get the metformin medication sponsored.

16 CLINICAL STUDY REPORT AND PUBLICATION POLICY

A summary report of the study will be provided to the CTIS portal within a year after the end of the trial.

17 REFERENCES

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