PROTOCOL

The metabolic effects of endocrine therapy (ET) in postmenopausal women with oestrogen-receptor-positive breast cancer

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CONFIDENTIAL

Statement of Compliance

This document is a protocol for a research project. This study will be conducted in compliance with all stipulation of this protocol, the conditions of the ethics committee approval, the NHMRC National Statement on Ethical Conduct in Human Research (2007) and the Note of Guidance on Good Clinical Practice (CPMP/ICH-135/95).

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STUDY SYNOPSIS

Title:	The metabolic effects of endocrine therapy (ET) in postmenopausal women with oestrogen-receptor-positive breast cancer
Design:	Single centre, prospective, controlled, observational study
Study Centres:	Austin Health and Heidelberg Repatriation Hospital
Hospital:	Austin Health and Heidelberg Repatriation Hospital
Study Question:	What are the biological effects of ET compared to controls (DCIS/no ET) on 1. Visceral adipose tissue, subcutaneous adipose tissue and total body fat 2. Other biomarkers of cardiovascular disease
Study Objectives:	1. To assess the effects of ET on visceral adipose tissue, subcutaneous adipose tissue and total body fat 2. To determine any associations between ET-related oestradiol depletion and increased CV burden 3. To identify possible mechanisms of how ET may contribute to CV disease
Primary Endpoints:	**1.** Changes in visceral adiposity tissue (measured by DXA) at 12 months in women on AI compared to controls **2.** Changes in subcutaneous adipose tissue and fat mass (measured by DXA) at 12 months in women on AI compared to controls
Secondary Endpoints:	• Changes in body composition in women on tamoxifen at 12 months compared to controls • Changes in peripheral microvascular endothelial function (measured by EndoPAT) at 12 months in women on ET compared to controls • Changes in insulin resistance (HOMA-IR, fasting glucose, insulin, c peptide and HbA1c) at 12 months in women on ET compared to controls

	• Changes in abdominal aortic calcification (measured by DXA) at 12 months in women on ET compared to controls • Changes in liver steatosis/fibrosis (measured by fibroscan) at 12 months in women on ET compared to controls • Changes in quality of life (Questionnaires and accelerometer readings) at 12 months in women on ET compared to controls
Inclusion Criteria:	**Women must meet all inclusion criteria to participate:** - Postmenopausal women with oestrogen-receptor positive non-metastatic breast cancer (TxMxM0) based on documented pathological and radiological evaluation - Ages 50-85 years (post-menopausal) **Endocrine Therapy group:** - Set to commence endocrine therapy as determined by the treating oncologist/commenced ET for no more than 8 weeks prior to recruitment **OR** **Control group:** -Ductal carcinoma insitu (DCIS) – any grade not on ET/ Early stage, non-metastatic breast cancer not requiring ET - Treatment intended for at least 12 months - Eastern Cooperative Oncology Group (ECOG) 0 and 1 - Able to independently read and understand the participant information and consent form and provide written, signed and dated data informed consent to participate in the study - Able and willing to meet all protocol required procedures and visits
Exclusion Criteria:	- Cessation or switching ET during the 12 month study period

	- Previous hormone replacement therapy within 12 months of study recruitment -Glucocorticoid use (continuously for ≥2 weeks within the 6 months prior to screening) - Malignancy within the past 5 years including breast cancer requiring treatment (except non-melanoma skin cancers) - Established type 2 diabetes mellitus - Any disease which is likely to lead to serious illness or death within the study period - Self reported recreational drug use or alcohol dependence within 12 months of screening
Number of Planned Participants:	130
Statistical Methods:	• Longitudinal changes in the primary and secondary endpoints will be analyzed using paired-sample t-tests, generalised linear mixed models and generalised estimating equations and compared between patients with and without endocrine therapy • Adjustments will be made for age, comorbidities and baseline measurements
Subgroups:	Tamoxifen Chemotherapy ER+ and HER2+ disease – commencing Herceptin as well as endocrine therapy Premature menopause/hysterectomy (<40yrs)

1. GLOSSARY OF ABBREVIATIONS & TERMS

Abbreviation	**Description (using lay language)**
AI	Aromatase inhibitor
BCa	Breast cancer
BMI	Body mass index = weight(kg)/height $(m)^2$
CVD	Cardiovascular disease
DCIS	Ductal carcinoma in situ

DXA	Dual energy X-ray absorptiometry
E1, E2	Oestrone, Oestradiol
ECOG	Eastern Cooperative Oncology Group performance score
ER-positive	Oestrogen-receptor-positive
FFM	Fat free mass
FM	Fat mass
HbA1c	Haemoglobin A1c
HDL	High density lipoprotein
HER 2 positive	Human epidermal growth factor receptor 2
HOMA-IR	Homeostatic model assessment of insulin resistance
LDL	Low density lipoprotein
OS	Ovarian suppression
SD	Standard deviation
SAT	Subcutaneous adipose tissue
TAM	Tamoxifen
TC	Total cholesterol
TG	Triglyceride
VAT	Visceral adipose tissue

STUDY SITES

Site	Address	Contact person	Phone	Email
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3. LAY SUMMARY & SCIENTIFIC BACKGROUND

3.1 LAY SUMMARY

Cardiovascular disease remains the highest cause of death in postmenopausal breast cancer survivors. This study aims to observe the effect of endocrine therapy, a class of commonly prescribed breast cancer medication on indirect markers of cardiovascular health.

We hypothesise that aromatase inhibitor use will increase visceral adipose tissue and total fat mass. It will also increase total cholesterol, glucose and increase risk factors of atherosclerosis including increase aortic calcification scores, and be associated with dysfunction of blood vessels. Finally, it will increase hepatic steatosis and negatively impact on mood and exercise levels. If these associations are observed, this project will provide a basis for future studies to address and mitigate these unfavourable changes in an already high cardiovascular risk population.

3.2 SCIENTIFIC BACKGROUND

3.2.1 Oestrogen Deprivation Therapy in Postmenopausal Women with ER-positive Non-Metastatic Breast Cancer

(i) Oestrogen deprivation therapy: risk-benefit ratio and clinical implications

Breast cancer is the most common cancer to be diagnosed in women worldwide (1). In Australia it is estimated that 18,087 women will be diagnosed with breast cancer. This is approximately 50 women per day with roughly 70% classified as having oestrogen receptor positive (ER+) disease and require adjuvant endocrine therapy (2). Despite the large numbers of postmenopausal women diagnosed, advances in management has led to

significant increases in cancer survival, with non- breast cancer deaths comprising 73% of the total mortality (3). Of these, cardiovascular disease (CVD) remains the major contributor to mortality.

Aromatase inhibitor (AI) therapy for 5 years is standard treatment for early stage breast cancer and has been reported to prolong disease-free survival when compared with the selective oestrogen receptor inhibitor tamoxifen, in postmenopausal women (4, 5). The aromatase enzyme catalyzes the rate limiting step in the conversion of androgen to oestrodial (E2). AIs profoundly inhibit aromatase (>95%) and therefore cause "virtually complete" E2 deficiency, with reduction of plasma E2 levels to 3 pmol/l or less (the detection limit of the most sensitive assays), markedly below postmenopausal levels of 20-50 pmol/L (6). In high cancer-risk women, extending AI therapy to 10 years or extending tamoxifen therapy with a further 5 years of AI therapy may decrease rates of new and recurrent cancers (5). However, a decrease in high density lipoprotein by up to 15% at 2 years and an increase in low density lipoprotein by up to 16.5% at 12 months in postmenopausal women on AI therapy has been reported when compared with tamoxifen (7, 8). Therefore, in women exposed to extended AI therapy, the benefits of decreased recurrence must be weighed against the associated accumulated risks of metabolic syndrome and CVD.

Unfortunately, CVD can be asymptomatic until an event occurs. For the growing cohort of postmenopausal breast cancer survivors on ET, there are currently no methods or guidelines to assist with stratifying or monitoring individuals at higher risk of CVD events. Therefore, there is little to guide physicians on duration or appropriateness of endocrine therapy (ET), particularly AI and extended AI therapy. Postmenopausal women are already at risk for metabolic syndrome and CVD. Along with increased age-related comorbidities such as hypertension and hyperlipidemia, reduced E2 levels have been associated with an increased risk for visceral fat accumulation – a clinically proven biomarker for CVD (9). Therefore, a pertinent clinical question is whether postmenopausal women with ER+ disease with E2 deprivation via ET therapy are at even higher risk for visceral fat accumulation and therefore future CVD events than women with residual postmenopausal levels of E2.

Body composition and biomarkers of CVD such as glucose metabolism, liver steatosis and peripheral microvascular endothelial function are readily available methods of potentially assessing individual CVD risk burden in postmenopausal women whilst on ET. If validated, these tests may serve an important role in guiding clinicians in personalising their decisions for duration of ET and extending AI therapy based on measurable markers of metabolic risk and CVD burden. Furthermore, these tests can ultimately be used to predict and proactively mitigate the long- term effects of ET therapy, including CVD events in those at higher risk and where extended AI therapy should be avoided or used with caution.

(ii) Oestrogen deprivation therapy as a model of oestrodial depletion in which to study the biological actions of residual E2 in postmenopausal women

In humans, oestradiol production is predominantly by the ovaries with a relatively smaller but clinically significant amount produced via aromatisation in peripheral tissues. ER-positive breast cancer is oestradiol sensitive and treatment with AI therapy in already postmenopausal women renders the body oestradiol deplete. Treatment with AI alone or with ovarian suppression (in pre-menopausal women) represents the only situation where an ethical requirement for oestradiol replacement is absent for prolonged periods of time and therefore these women offer a unique model of profound untreated oestradiol depletion over an extended period.

3.2.2 Effects of Profound Oestradiol Deprivation on Body Composition

The distribution of fat, more so than BMI, is what has been clinically correlated with the development of cardiometabolic disturbances. Increased visceral adipose tissue (VAT), is not only associated with the multiple risk factors for CVD, such as insulin resistance, hypertriglyceridemia, hypertension and chronic inflammation, but is also known to independently increase risk of CV events (12) – making it a significant risk factor that potentially can be targeted and reversed.

Changes in waist circumference and body composition, including reductions in lean body mass and an increased propensity for weight gain and increased fat VAT have been associated with the menopausal state (9,10). Replacement of E2 via menopausal hormone therapy is associated with reduced total and visceral adiposity, suggesting these changes in body composition are the result of E2 deficiency rather than of age per se (11).

To date, the effect of "virtually complete" E2 deprivation on body composition remains unclear. There are only a few studies examining the effects of E2 deprivation as a result of AI treatment on body composition in postmenopausal women, with the only randomized controlled trials in the current literature comprising mainly of small studies (ranging from 56 to 82 participants) with participants having had previous exposure to tamoxifen and/or adjuvant chemotherapy (7, 8, 13). Control groups were exposed to ongoing tamoxifen which is an important confounder of changes to body composition and lipid profile. The only observational study describing the effects of AI therapy on VAT observed an increase in visceral adipose tissue in women treated with AI therapy for 6 months compared to baseline. The retrospective study had small numbers (n=64), and importantly lacked a control group. Moreover, participants had previous exposure to chemotherapy which in itself may affect body composition due to effects on food intake, activity levels and concomitant glucocorticoid therapy (14). There are currently no prospective studies that investigate the effects of profound E2 deprivation as a result of AI therapy alone. Furthermore, apart from the previously described retrospective uncontrolled, observational study, none of the current studies have assessed VAT.

While guidelines recommend 1-2-yearly DXAs for women on AI therapy to monitor bone health (15), body composition by DXA can be performed simultaneously at no extra cost, and with significantly less radiation than standard computerised tomography (CT). We have locally validated that body composition measured by DXA, including VAT is highly correlated with traditional gold standards of magnetic resonance imaging and CT (16). If an association of AI therapy with increased VAT, subcutaneous adipose tissue (SAT) and total percentage fat mass is observed, body composition along with waist measurements may be valuable surveillance markers for metabolic syndrome and CVD burden that would assist with clinicians' decision regarding ET therapy type and duration.

3.2.3 Effects of Profound Oestradiol Deprivation on Biomarkers of Glucose Metabolism and Dysipidemia

Whilst it is controversial whether VAT is directly pathogenic or a consequence of limited expandability of SAT leading to metabolically harmful ectopic fat distribution, increased VAT is associated with deleterious changes in inflammatory markers and adipokines including leptin and adiponectin (9) and therefore, at minimum a clinically proven marker of metabolic and CVD risk. Furthermore, individuals with known metabolic syndrome have been shown to display significantly higher levels of leptin, insulin, HOMA-IR (a validated measure of insulin resistance) and dyslipidaemia (17).

Given the increase in VAT and insulin resistance in postmenopausal women, determining the biological effects of profound E2 deprivation on these inflammatory markers and markers of glucose metabolism is important. If a correlation is identified between these markers and ET use, they can potentially be used in addition to body composition as biomarkers of metabolic syndrome to assist in stratifying CVD risk. Women identified as "high CVD risk" can then be managed pre-emptively for modifiable risk factors.

3.2.4 Effects of Profound Oestradiol Deprivation on abdominal aortic calcification and endothelial function

Although there are currently minimal RCTs examining the direct effects of oestradiol deprivation from endocrine therapy on endothelial function or abdominal aortic calcification, human and animal data suggest that E2 is protective against atherosclerosis. The athero-protective effect of E2 however, appears to be limited to the endothelium via inhibition of atherosclerotic lesion formation (18). In mice studies, E2 does inhibit lesion formation, but did not alter the progression of established atherosclerotic lesions (19). The Early versus Late Intervention Trial with Estradiol (ELITE) (18), a single-centre, randomised, double-blinded, placebo-controlled trial involving 643 postmenopausal women, showed that when hormone therapy (HT) was initiated ≥6 years of menopause, HT significantly reduced the progression of subclinical atherosclerosis (measured by carotid artery intima-media thickness (CIMT)). This was in contrast to women treated ≥10 years since menopause where no effect on the progression of atherosclerosis was observed. In a secondary analysis of the ELITE study (29), E2 levels were inversely associated with CIMT progression in early postmenopausal women (who were ≤6 years of menopause) and were positively associated with CIMT progression in late postmenopausal women (who were ≥10yrs post-menopause)

Digital arterial tomography via EndoPAT is validated and non-invasive method of measuring peripheral microvascular endothelial dysfunction. The EndoPAt index has been demonstrated to have a significant inverse relationship to multiple cardiovascular risk factors and is both sensitive and specific at diagnosing early coronary atherosclerosis (20, 21).

Abdominal aortic calcification scores have been less well studied, but have been correlated with carotid ultrasound measurements of atherosclerosis and appears to be a promising marker of generalised extra-coronary atherosclerosis.

3.2.5 Effects of Profound Oestradiol Deprivation on hepatic steatosis

The adverse hepatic effects of tamoxifen have been well described with increased risk of hepatic steatosis. The effects of AIs on the liver is less well described, with a recent observational cohort study suggesting an increased risk of fatty liver in tamoxifen users compared to AI users (22).

Fibroscan is a non-invasive ultrasound-based method that uses shear wave velocity to assess tissue. Among the non-invasive tests, transient elastography (FibroScan®, TE) with controlled attenuation parameter (CAP) has demonstrated good accuracy in quantifying the levels of liver steatosis and fibrosis in patients with non-alcoholic fatty liver disease (NAFLD), the factors associated with the diagnosis and NAFLD progression. The method is fast, reliable and reproducible, with good intra- and interobserver levels of agreement, thus allowing for population-wide screening and disease follow-up (23).

NAFLD is not only a marker of metabolic syndrome, but also a risk factor for CVD. The clinical implication of these findings is that NAFLD patients may benefit from more intensive monitoring and early therapeutic interventions to lower the risk of cardiovascular disease.

3.2.4 Effects of Profound Oestradiol Deprivation on Mood and Behaviour

Studies reviewing psychological side effects are few and are conflicting in results. One uncontrolled study reviewed breast cancer survivors of whom 37 % were treated with hormonal therapy and reported one-third experienced a multifactorial psychological interference with their abilities to cope with their diagnosis, symptoms and treatment (24). Another study evaluated symptoms of depression in breast cancer patients treated with tamoxifen or exemestane and compared to healthy controls. Researchers indicated that there were no differences between patients and control groups with regard to mental health or depressive symptoms (25). Our study aims to assess the changes in mood and behavior in women on AI therapy when compared to a diagnostically comparable breast control group. While use of a control group of untreated women with breast cancer is not feasible, using women with a diagnosis of DCIS or early stage breast cancer not requiring ET, removes to some extent the important confounder of a recent cancer diagnosis.

Furthermore, although E2 deprivation has been reported in preclinical studies to directly impact on multiple somatic tissues including adipocytes to alter body composition and glucose metabolism, central nervous mechanisms that may lead to decreased energy levels or increased food intake either directly or secondary to behavioural effects of mood remains unclear in profound E2 deplete women. E2 is involved in the regulation of appetite and energy expenditure, and its elimination in rodent studies resulted in increased food intake and decreased ambulatory and wheel running activities, which was reversed with estrogen replacement (26, 27).

Physical Activity Frequency and Quality of Life assessment tools will shed light on the impact of profound E2 deprivation on the lifestyle, mood and exercise frequency of postmenopausal women on endocrine therapy and the potential indirect role it plays on body composition and therefore metabolic factors and cardiovascular health. If an association is observed, it would provide a basis for future targeted interventional studies to address and mitigate this mood and behavioural risks associated with endocrine and particularly AI therapy.

3.3 NOVELTY AND CLINICAL SIGNIFICANCE

Our study will be a 24-month prospective, controlled, observational study investigating the biological effects of endocrine therapy, in particular, the profound oestradiol deprivation from endocrine therapy on body composition in oestrogen receptor positive, postmenopausal women. Controls will include aged-matched postmenopausal women with breast ductal carcinoma in-situ. The current mechanisms of how endocrine therapy, particularly aromatase inhibitors may affect cardiovascular risk remains unknown.

We hypothesize that endocrine therapy and its effects on oestradiol deficiency will lead to an increase in VAT, SAT and total body fat, along with other unfavourable changes on biomarkers of metabolic health including glucose metabolism, lipid profile, degree of liver steatosis, peripheral microvascular endothelial dysfunction and increased abdominal aortic calcification.

With cardiovascular disease being the highest cause for mortality in postmenopausal breast cancer survivors, having an easily accessible means of quantifying and measuring cardiovascular risk that can then be incorporated into the routine monitoring for these high-risk women is essential.

If deleterious changes in body composition and along with biomarkers of cardiovascular burden are observed with endocrine therapy, measures of these indirect biomarkers can be used to personalise endocrine therapy. By measuring these biomarkers, clinicians will be better able to stratify each patient's cardiovascular risk. Women identified as "high cardiovascular risk" can be managed pre-emptively for modifiable risk factors and the risk versus benefits of commencement and duration of endocrine therapy – particularly AI therapy, can therefore be individualised.

Furthermore, if an association between endocrine therapy and metabolic health is observed, this project will provide a basis for future targeted interventional studies to address and mitigate these biological changes that are indirect markers of cardiovascular risk.

4. STUDY OBJECTIVES

4.1 STUDY AIMS

- To assess the effects of oestradiol depletion from endocrine therapy, in postmenopausal women with oestrogen-receptor-positive breast cancer on visceral adipose tissue, subcutaneous adipose tissue and total fat mass (as measured by DXA)

- To determine if an association exits between endocrine therapy -related oestradiol depletion and increased cardiovascular burden as measured by the following biomarkers:

    1. Peripheral microvascular endothelial function (measured by EndoPAT index)
    2. Extra-coronary atherosclerosis (measured by DXA and abdominal aortic calcification scores)
    3. Degree of hepatic steatosis (measured by fibroscan)
    4. Insulin resistance (measured by HOMA-IR, fasting glucose and HbA1c)
    5. Dyslipidemia (measured by fasting lipids)

- To identify possible mechanisms of how endocrine therapy may contribute to cardiovascular disease

4.2 HYPOTHESES

Aromatase-inhibitor-related oestradiol depletion will, when compared to maintenance of low, but residual postmenopausal oestradiol levels, be associated with increased visceral adipose tissue, subcutaneous adipose tissue and total fat mass.

4.3 OUTCOME MEASURES

Patients receiving each form of endocrine therapy (AI, tamoxifen, control) will be compared across groups with respect to the following primary and secondary endpoints.

1. **Primary Endpoints**
    - Changes in visceral adiposity tissue at 12 months in women using aromatase inhibitors versus controls (DCIS/early stage breast cancer not requiring ET) as measured by DXA

Changes in subcutaneous adiposity tissue, fat and lean mass at 12 months in women using aromatase inhibitors versus controls (DCIS/early stage breast cancer not requiring ET) as measured by DXA

2. **Secondary endpoints:**
   - Changes in body composition in women using tamoxifen at 12 months (measured by DXA, hologic) versus controls (DCIS/early stage breast cancer not requiring ET)
   - Changes in glucose metabolism (measured by HOMA-IR, fasting glucose, insulin, c peptide, HbA1c and adiponectin, leptin levels) at 12 months
   - Changes in lipid metabolism (measured by serum lipids) at 12 months
   - Changes in abdominal aortic calcification (measured by DXA) at 12 months
   - Changes in peripheral microvascular endothelial function (measured by EndoPAT) at 12 months
   - Changes in degree of hepatic steatosis (measured by fibroscan and liver function tests) at 12 months
   - Changes in visceral and subcutaneous abdominal fat volume and distribution (anthropometric measurements) at 12 months
   - Changes in behaviour, mood and lifestyle (Assessment tools to determine any significant changes in appetite and exercise) at 12 months
   - Changes in sex steroids at 12 months
   - Changes in bone remodelling markers (ctx, p1np) at 12 months

5. STUDY DESIGN

5.1 STUDY TYPE & DESIGN

A prospective, controlled, observational study will be conducted at a single centre to examine the effects of 12 months of aromatase therapy on body composition, and other biomarkers of CVD burden. A total of 100 patients will be recruited. A separate group of 30 patients receiving tamoxifen therapy will also be recruited to serve as a comparator group for analysis of secondary endpoints.

5.2 SAMPLE SIZE CALCULATION

In studies reporting data on visceral adiposity in postmenopausal women using DXA, the standard deviation of patients' visceral adiposity ranged between 15-100cm^2 (14, 16,29, 30, 31).

As such, for the primary endpoint of change in visceral adiposity as in this study, we consider a 25% difference in visceral adiposity a clinically significant difference for this

study. As such, to detect a 25% difference in visceral adiposity tissue, assuming a standard deviation of $40cm^2$, a power of 80% and a two-sided significance level of 0.05, we will require recruitment of 40 participants in each study group. Factoring in a drop-out rate of 30%, we will require 100 participants in total.

With a sample size of 100, the study is adequately powered to detect a 25% difference in visceral adiposity tissue, assuming a standard deviation of $40cm^2$, a power of 80% and a two-sided significance level of 0.05. This number accounts for 30% attrition. Study recruitment is progressive so to ensure that the study will be adequately powered and so withdrawals can be accounted for.

Therefore, we aim to recruit a total of 100 patients (50 in each arm).

5.3 RECRUITMENT PROCEDURE

This trial will recruit from a population of postmenopausal women being treated at Austin Health for ER-positive breast cancer. Specifically, we will be recruiting from our multi-disciplinary Adjuvant Endocrine Breast Cancer Clinic that manages all women with breast cancer commenced on endocrine therapy.

We will take referrals of eligible women from Austin Health Physicians in private practice that are performing an analogous role to that of the public Adjuvant Endocrine Breast Cancer Clinic. If not already patients of Austin Health, these participants will receive an Austin Health unit record number. Health professionals who are known to have significant contact with patients who have breast cancer will be contacted to be made aware of the study and will be provided with information and the contact details of the investigators should they think a patient may fit the eligibility criteria.

The recruitment of 50 patients on AI and 50 DCIS/early breast cancer not requiring ET controls will be performed as per the sample size calculations (above).

In addition, we aim to recruit 30 patients receiving tamoxifen therapy for breast cancer so as to use this subset as a group with which to perform secondary endpoint analyses. Based on clinical experience and audit, this is the expected number of cases over the time course (18 months) of the recruitment phase of this study.

5.4 INCLUSION AND EXCLUSION CRITERIA

*INCLUSION CRITERIA

Women must meet all inclusion criteria in order to participate in the study.

- Postmenopausal women with oestrogen-receptor-positive, non-metastatic breast cancer (TxNxM0) based on documented pathological and radiological evaluation

- Age 50-85 years
- To commence adjuvant treatment with endocrine therapy as determined by treating oncologist/commenced endocrine therapy for no more than 8 weeks prior to recruitment
  OR
- Postmenopausal women with DCIS (any grade)/early breast cancer not requiring ET
- Endocrine therapy intended for at least 12 months
- ECOG 0 and 1
- Able to personally read and understand the Participant Information and Consent Form and provide written, signed and dated informed consent to participate in the study
- Able and willing to meet all protocol-required procedures and visits

Perimenopausal: absent cycles for 3-12months

Postmenopausal: absent cycles for 12 months

*EXCLUSION CRITERIA

Current evidence or prior history of any of the following:

- Malignancy within the past 5 years including breast cancer requiring previous treatment (not including non-melanoma skin cancer)

-Cessation or switch of endocrine therapy during the first 12 months

-Previous hormone replacement therapy within 12 months of study recruitment

-Established type 2 diabetes mellitus

-Self reported recreational drug use or alcohol dependence within 12 months prior to screening

-Any disease which is likely to lead to serious illness or death within the study period

5.5 CONSENT

Prior to study entry which includes screening for eligibility, participants who wish to take

part in the trial will have a detailed verbal discussion with a study doctor regarding all details of their participation in the trial. This will involve details about the imaging studies to be undertaking, blood collection procedures, storage, analysis (including potential future genetic testing), and publication of results. All this information will be available in in writing via the Participant Information Consent Form (PICF). Participants will be able to take this form home and discuss with their relatives/friends/local health worker if they wish prior to deciding whether or not to take part in the study. They will be allowed to ask questions regarding any information in the PICF. After this, the participant is willing to take part in the

study, the participant will sign the Consent Form in the PICF to acknowledge that they have given informed consent. No part of the study will be undertaken until the participant signs the PICF. The participant will be given a copy of the PICF to keep for their records.

Individual informed consent will be obtained from each participant prior to any part of the study being undertaken. Participants will be provided with a copy of the signed Participant Information Consent Form. It will be explicit from the verbal and written consent process and throughout the study, the participation is voluntary, and that withdrawal is allowed. It will also be made very clear that their treatment in the clinic and their relationship with their doctors will not be affected by participation or non-participation.

6. STUDY METHODOLOGY

6.1 STUDY VISITS

The following is a list of study interactions/visits and the data that will be collected. For the data collected please see section '6.3' for the specific measurements and techniques used.

(i) Screening of potential participants who consent:

- Medical interview and review of Austin Health medical file: demographic data (name, age, date of birth, hospital unit record number), and medical history and medication history sufficient to determine trial eligibility
- For eligible participants, screening blood test and DXA scan will be used as baseline measurements if obtained within 8 weeks prior to entry into the trial
- Patients are eligible for a baseline assessment if they plan to commence ET within 8 weeks of the assessment

(ii) Visit 1: Further baseline assessment of participants who are eligible based on screening

- Bloods tests
- Medical Assessment and Anthropometric data
- Questionnaires
- DXA scan – measurements for bone mineral density/trabecular bone score, body composition and aortic calcification will be undertaken
- EndoPAT peripheral arterial tonometry
- Fibroscan

(iii) Visit 2: 6 months post recruitment

- Blood tests
- Medical Assessment and Anthropometric data

- Questionnaires
- DXA
- EndoPAT peripheral arterial tonometry
- +/- Fibroscan (only if trends in liver function tests to suggest worsening steatosis or fibrosis)

(iv) Visit 3: 12 months post recruitment

- Blood test
- Medical Assessments and Anthropometric data
- Questionnaires
- DXA
- EndoPAT peripheral arterial tonometry
- Fibroscan

(v) Visit 4: 24 months post recruitment

- Blood test
- Medical Assessments and Anthropometric data
- Questionnaires
- DXA
- EndoPAT peripheral arterial tonometry
- Fibroscan

6.2 STUDY SCHEDULE

Visit month	Event	Time (hrs)	Assessments	Standard Care	Dosage/Volume (blank where N/A)
Informed consent					
0	Screening visit	1	Medical interview	X	
0	Baseline Assessment	3	Medical assessment and Anthropometric data (1hr)	X	
			Blood tests (30min)	X	22.5ml standard care 20ml additional for study
			DXA** (30-45min) (whole body composition, trabecular bone score and abdominal aortic calcification)	X	0.06mSv
			EndoPAT (15 min)		
			Fibroscan (10min)		
			Questionnaires (10min)	X	

3	No visit	0	Blood tests	X	22.5ml standard care 20ml additional for study
6	Study visit 6 months from baseline	3	Medical assessment and Anthropometric data	X	
			EndoPAT		
			+/- Fibroscan		
			Questionnaires	X	
			Blood tests	X	22.5ml standard care 20ml additional for study
			DXA**		0.06 mSv
12	Study visit 12 months from baseline	3	Medical assessment and Anthropometric data	X	
			EndoPAT		
			Fibroscan		
			Questionnaires	X	
			Blood tests	X	22.5ml standard care 20ml additional for study
			DXA**	X	0.06 mSv
24	Study visit 24 months from baseline	3	Medical assessment and Anthropometric data	X	
			EndoPAT		
			Fibroscan		
			Questionnaires	X	
			Blood tests	X	22.5ml standard care 20ml additional for study
			DXA**	X	0.06 mSv

* Assessment without an 'X' are in addition to standard care

** Assessment of total body composition and abdominal aortic calcification are in addition to standard care, which involves scanning of the hip and spine

6.3 TESTS, MEASUREMENTS AND TECHNIQUES

6.3.1 Body composition by DXA

Visceral adipose tissue, subcutaneous adipose tissue, total body fat and lean body mass will be studied by DXA (Hologic with Apex 5.6 software). Bone mineral density and trabecular bone scores will also be measured as part of standard care.

6.3.2 Laboratory measurement of glucose and lipid metabolism

Leptin, adiponectin, HOMA-IR, HbA1c, fasting glucose, insulin, c peptide and lipids will be measured.

6.3.3 Laboratory measurement of estradiol and estrone

Oestradiol and estrone will be measured by a validated sensitive mass spectrometry (28). If further precision is required, an ultrasensitive estradiol method with a limit of qualification (LOQ) of 0.25pg/ml with accuracy (93-110%) and precision (median coefficients of variation 4%, all concentrations above LOQ <15%). The Austin Health researchers will be collaborating with Professor David Handelsman from the ANZAC Research Institute in Sydney to enable very accurate measurement of hormone levels in the blood samples

6.3.4 Mood and exercise frequency by validated questionnaires and accelerometer measurements

Mood will be assessed via the Functional Assessment of Cancer Therapy-Breast (FACT-B) and Menopause-Specific Quality of Life (MENQOL) tools and physical activity levels via the accelerometer measurements.

6.3.5 Abdominal aortic calcification by DXA

Abdominal aortic calcification will be calculated using the lateral spine images acquired via DXA. The Abdominal aortic calcification score calculated will then be interpreted.

6.3.6 Endothelial dysfunction via EndoPAT

Peripheral microvascular endothelial dysfunction will be measured via the EndoPAT device (endo-PAT2000 version 3.2.4; Itamar Medical Ltd, Caesarea, Israel). All women including those with previous axillary lymph node or sentinel lymph node surgery will be included, but their surgical arm will be used as a control. Patients should be fasted prior to testing but can have water. Medications that affect vascular tone should be withheld that morning. A baseline blood pressure done on the surgical arm will be used as a control blood pressure. This control blood pressure (BP) will determine how high the systolic blood pressure on the experimental (nonsurgical) arm will need to be increased to (200mmHg or 60mmHg above the systolic – whichever is higher). There is currently no strong evidence in the literature for increased lymphoedema risk with the measurements of blood pressure in these women. This has also been discussed at the Austin Health Oncology multidisciplinary meeting where the consensus between oncologists and surgeons is that blood pressure measurements on the side of previous axillary lymph node surgery is acceptable. Arm circumference measurements will be monitored throughout the study. Patients with a previous known diagnosis of lymphoedema will not have blood pressure measurements performed on the affected arm and the target BP will be set at 200mmHg in the experimental arm.

6.3.7 Hepatic steatosis via fibroscan

Fibroscan will be used to determine steatosis/fibrosis. Patients are required to be fasting prior to the procedure. All patients with abnormal findings will be referred on to the gastroenterology clinic.

6.3.8 Anthropometric data

This will include clinical measurements of height, weight, BMI, waist circumference, hip circumference and waist:hip ratio.

6.3.9 Routine Bloods

Baseline: FBE, UEC, CMP, LFT, AST, ctx, p1np, 25 OH Vit D, oestradiol, FSH, LH, IGF-1, SHBG, PTH, CRP, TSH, FT3, FT4, IgA, Anti-gliadin ab, TTG Ab

Review: FBE, UEC, CMP, LFT, AST, ctx, p1np, 25 OH Vit D, oestradiol, FSH, LH IGF-1, SHBG, PTH, CRP

6.4 Follow up

All patients will be followed up for a total of 24 months over a total of 4 study visits.

7. PARTICIPANT SAFETY AND WITHDRAWRAL

7.1 RISK MANAGEMENT AND SAFETY

Participants will be informed that there is a risk that performing some of the tests may distress them, either physically or emotionally. Fully trained staff will perform all tests which will minimise this risk. There may be additional risks that researchers do no expect. Any adverse events will be managed at Austin Health and would be free of charge as a public patient.

1. Possible risks associated with ionising radiation

This research study involves exposure to a very small amount of radiation. Compared to background radiation exposure of 2 millisiverts (mSv) per year, the effective dose from this study is approximately 0.24 mSv. At this dose level, no harmful effects of radiation have been demonstrated, as any effect is too small to measure. The risk is believed to be minimal.

2. Possible risks associated with blood tests

Venepuncture can cause some discomfort and bruising. Infection is extremely rare following venepuncture.

3. Discomfort associated with inflation of blood pressure cuff during measurements of peripheral arterial microvascular function

Blood pressures will be done with the aim to reach 200mmHg or 60mmHg above the patient's systolic blood pressure. This can cause some discomfort and temporary paraesthesia. These blood pressures will only be performed on the non-surgical arm with a

normal blood pressure to be performed on the surgical arm. Patients with known lymphoedema will not have a blood pressure performed on the surgical arm.

4. Psychological distress

We do not envisage that this study will cause ay participant psychological distress. If participants do experience distress, the will be encouraged to speak to the study doctors. An appropriate level of care will be triaged by the study doctors. This may include withdrawal from the study, referral to a general practitioner or Austin Health psychologist/psychiatrist for psychological support.

7.2 HANDLING OF WITHDRAWALS

Participants who wish to withdraw from the study or who need to be withdrawn for other reasons will be reviewed by a study doctor to discuss this. It will be clear to them throughout the trial at that time, that withdrawal will not negatively impact their relationship with their treating doctor, or Austin Health. No additional personal, blood samples or health information will be collected from participants who withdraw.

8. STATISTICAL METHODS

8.1 STATISTICAL METHODS TO BE UNDERTAKEN

Longitudinal changes in the primary and secondary endpoints will be analyzed using paired-sample t-tests, generalised linear mixed models and generalised estimating equations and compared between patients with and without AI therapy. Adjustments will be made for age, comorbidities and baseline measurements.

9. STORAGE OF BLOOD AND TISSUE SAMPLES

9.1 DETAILS OF WHERE SAMPLES WILL BE STORED, AND THE TYPE OF CONSENT FOR FUTURE USE OF SAMPLES

All laboratory studies aside from sex steroid analyses (using LC-MS) will be performed at Austin Pathology on the same day that blood is collected from participants. The results of these tests will go into the participant's Austin Health medical record and be collected separately in their study file. Blood will be discarded within 14 days of analysis.

Blood for sex steroid analysis will be collected on the same day but will be spun, aliquot and stored in a locked freezer in the Austin Health Endocrine Department laboratory at -80 degrees Celsius. Blood samples will be stored by study number only and external researchers/organisations will not be able to identify participants by this number. At study completion, an aliquot from each participant, from each time point, will be sent as a batch

to the ANZAC Research Institute for sex hormone analysis. Blood samples will be discarded by the ANZAC Research Institute within 14 days of analysis.

If participants give their consent for their blood to be stored for future ethically approved research +/- genetic testing (unspecified consent), the other frozen aliquots will be maintained in a locked Endocrine Department Laboratory freezer indefinitely, for future ethically approved research into disease related to Women's Health. These samples will only be labelled with study number. This freezer is locked and managed by a research scientist. Access to these blood samples is only by certified laboratory technicians employed by the Endocrine Department. Consent for this storage and future ethically approved research +/- genetic testing will be explicitly sought as detailed in the Participant Information Consent Form. For participants who do not provide unspecified consent for future ethically approved research +/- genetic testing, blood will only be stored for sex steroid analysis to be done at study completion.

All collected blood samples will be in an individually re-identifiable form. The code to match study number with identifying participant hospital record number and demographic details will be stored in an electronic file in a password protected area of a University of Melbourne Department of Medicine (Austin Health) server and only study doctors will have access to this file. Access to the University of Melbourne Department of Medicine server requires individual log in details and is controlled and monitored.

10. DATA SECURITY & HANDLING

10.1 DETAILS OF WHERE RECORDS WILL BE KEPT & HOW LONG WILL THEY BE STORED

Paper copy information will be stored in a locked filing cabinet within a locked office located in the University of Melbourne Department of Medicine, Austin Health. Each participant will have a file, which will be identified by study number only.

An electronic database file will also be kept for each participant as backup and as a way to analyse this information. This will also be identified by study number only and stored on a password-protected file on a University of Melbourne Department Medicine Server.

Electronic and paper study files will be stored for 15 years after the end of the study. After that time, electronic information will be permanently deleted. Paper records will be destroyed in Austin Health Confidential Waste bins.

10.2 CONFIDENTIALITY AND SECURITY

Paper copy information will be stored in a locked filing cabinet within a locked office located in the University of Melbourne Department of Medicine, Austin Health. Each participant will have a file, which will be identified by study number only.

An electronic database file will also be kept for each participant as back up and as a way to analyse this information. This will also be identified by study number only and stored on a password-protected file on a University of Melbourne Department of Medicine Server.

The code to re-identify participants will be kept in a separate password protected file on a Department of Medicine Server.

Access to the University of Melbourne Department of Medicine server requires individual log in details and is controlled and monitored.

10.3 ANCILLARY DATA

Images from scans will be stored in the participant's Austin Health medical record and in their electronic study file.

11. TIMELINE

Ethics approval for the study from the Austin Health HREC will be done in 2018. Recruitment will begin in early 2019 and we expect this to be completed within 18 months, allowing completion of final study visits at 42 months. The final 6 months of my will be dedicated to data analysis and submission of primary manuscript. We anticipate the results of this trial will be published in peer-reviewed medical literature. It will also form a component of a PhD thesis which will be submitted to the University of Melbourne by one of the associate investigators, Dr Yee-Ming Cheung.

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