Phase II Study of Metformin for Reduction of Obesity-Associated Breast Cancer Risk

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SCHEMA

Phase II Study of Metformin for Reduction of Obesity-Associated Breast Cancer Risk

Premenopausal women
(21– 54 yrs old; Overweight or obese; Metabolic syndrome)

↓

Screening
(Informed consent; Medical History; Anthropometric Measures; Fasting blood; Urine)

↓

Baseline Evaluation
(FWR-MRI; Fasting blood; NAF; Urine; Anthropometric Measures; AFFQ/AAFQ; Breast biopsy (optional))

↓

Randomization
(Metformin or Placebo; 75 per arm)

↓

Agent Intervention (12 months (52 ± 4 wks))
(1 tablet of metformin (850 mg) or placebo daily for the first 4 weeks and
2 tablets of metformin (850 mg) or placebo daily for the remaining intervention period)

↓

Month 3 Visit (13 ± 2 wks)
(Safety; Compliance; Urine)

↓

Month 6 Visit (26 ± 4 wks)
(FWR-MRI; Fasting blood; NAF; Urine; Anthropometric Measures; Safety; Compliance; Breast biopsy (optional))

↓

Month 9 Visit (39 ± 2 wks)
(Safety; Compliance; Urine)

↓

Month 12 Visit (52 ± 4 wks)
(FWR-MRI; Fasting blood; NAF; Urine; Anthropometric Measures; AFFQ/AAFQ)

Primary Endpoint:

- Changes in breast density assessed by FWR-MRI

Secondary Endpoints:

- Modulation of systemic hormones, growth factors, and cytokines (insulin, sex steroid hormone, IGF-axis, adipokines) and anthropometric measures
- Modulation of metabolomic profiles in NAF and plasma and correlations of metabolomic profiles with markers of breast cancer risk

Exploratory Endpoints:

- Tissue morphological changes
- Modulation of cellular and molecular targets of metformin in the breast tissue

Abbreviations used in schema: FWR-MRI: MRI-acquired fat-to-water ratio; NAF: nipple aspirate fluid; AFFQ: Arizona Food Frequency Questionnaire; AAFQ: Arizona Activity Frequency Questionnaire; IGF: insulin-like growth factor

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1. OBJECTIVES

The overall objective of this Phase II double-blind, randomized, placebo-controlled trial of metformin is to determine its potential effects on recognized and putative markers of breast cancer risk in overweight or obese premenopausal women who have metabolic syndrome.

Primary objectives:
To determine the effect of metformin intervention on breast density assessed by MRI-acquired fat-to-water ratio (FWR-MRI). Changes in breast density will be assessed and compared between metformin and placebo treated groups.

Secondary objectives:

- To determine the effect of metformin intervention on metabolic disturbances and body weight/composition. Changes in circulating levels of insulin, IGF axis, sex steroid hormones, adipokines, and anthropometric measures will be assessed and compared between treatment groups.

- To explore the application of metabolomics as a systems biology approach to assess the chemopreventive mechanisms of metformin. The global profiles of biochemicals and metabolites in nipple aspirate fluid (NAF) and plasma will be assessed through unbiased metabolome wide analysis to determine the metabolic changes induced by metformin in comparison with placebo. We will also explore whether metformin-induced metabolic changes correlate with metformin-induced changes in markers of breast cancer risk or side effects. In a cross-sectional fashion, we will explore the correlations between baseline metabolic features with breast density and circulating levels of hormones and cytokines.

Exploratory objectives:
To determine the effect of metformin intervention on tissue architecture as well as cellular and molecular targets in breast tissue collected in a subgroup of participants. The tissue level information will also allow us to conduct correlative studies with our primary endpoint and secondary endpoints. These analyses will provide the much needed insight on the potential mechanisms of metformin action at the tissue level and aid in validating FWR-MRI and nipple aspirate as surrogate measures for tissue changes with metformin intervention.

2. BACKGROUND

2.1 Significance

Obesity-associated breast cancer risk

A recent report indicates that two-thirds of the U.S. adults are overweight or obese (1). High adiposity is a major risk factor for a number of chronic diseases, including type 2 diabetes, cardiovascular diseases, and certain types of cancer, including postmenopausal breast cancer (2-5). The increased postmenopausal breast cancer risk in women with high adiposity is likely to be attributed to multiple metabolic disturbances including altered circulating sex steroid hormones, hyperinsulinemic insulin resistance, altered expression and secretion of adipokines from adipose tissue, increased production of pro-inflammatory cytokines, and increased oxidative stress.

Metformin for Reduction of Obesity-Associated Breast Cancer Risk Metformin, a widely used antidiabetic drug, exerts favorable effects on multiple metabolic disturbances which may lead to reduction of breast cancer risk in women with high adiposity. Metformin has been shown to reduce circulating insulin levels (reviewed by (6)), increase insulin-like growth factor binding protein (IGF-BP) 1 (7, 8), decrease serum testosterone and androstenedione levels (9, 10), and increase serum sex steroid binding globulin (9, 10) in non-diabetic women with polycystic ovary syndrome (POS). Reduction in serum insulin levels was also observed in non-diabetic breast cancer patients with metformin treatment (11). In diabetics, metformin treatment resulted in favorable changes in circulating levels of leptin and adiponectin (12, 13). Furthermore, studies have shown that metformin intervention resulted in weight reduction in overweight or obese patients without type 2 diabetes, although these trials were mostly small and of weak design and weight reduction was not observed in all studies (reviewed by (14)).

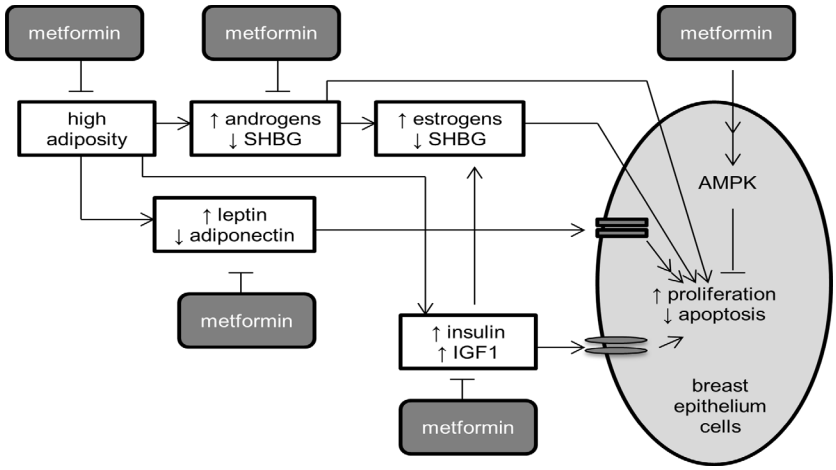

Figure 1. Simplified schematic of the potential mechanisms of action of metformin in prevention of obesity-associated breast cancer

In addition to the indirect effects, metformin may exert a direct effect in mammary tissue through the activation of the AMP-activated protein kinase (AMPK) signaling pathway, leading to an antiprolierative effect and induction of apoptosis (15-18). However, metformin is an organic cation at physiological pH. Its cellular uptake generally requires the presence of cell surface transporters. It is not known whether the human mammary tissue expresses the transporters for metformin to exert its direct effect.

Epidemiological and Clinical Studies

Case control and cohort studies investigating the relationship between diabetes and cancer found that treatment with metformin appears to substantially reduce the risk for development of cancer in diabetics (19, 20), including breast cancer (21-24). Given the retrospective nature of these studies and the possibility that the comparison treatments (such as sulfonylureas or exogenous insulin) may increase risk, randomized, placebo-controlled intervention trials are clearly needed to assess the breast cancer preventive activity of metformin. It is important to note that accumulating evidence suggests that type 2 diabetes and obesity share biological mechanisms for their association with breast cancer (reviewed by (25)). Therefore, metformin would have high potential for breast cancer risk reduction in non-diabetics with high adiposity.

Recent window-of-opportunity neoadjuvant trials reported clinical activity of metformin in non-diabetic women with operable invasive breast cancer after 2-4 weeks of metformin. In two small non-placebo controlled trials, short-term metformin intervention prior to surgery resulted in decreased tumor cell proliferation (26, 27). A considerably larger randomized, placebo-controlled trial did not show an overall decrease in tumor cell proliferation after 4 weeks of metformin intervention (28). However, subgroup analyses showed a decrease in tumor cell proliferation and serum insulin levels in women with high adiposity or insulin resistance,

suggesting the importance of considering the metabolic characteristics of the study population (28).

2.2 Study Design and Rationale

The overall objective of the proposed study is to evaluate the potential activities of metformin for breast cancer risk reduction in overweight or obese premenopausal women who have metabolic syndrome. This study population is at increased risk for postmenopausal breast cancer and has a high prevalence of metabolic disturbances. In addition, clinical and animal studies suggest that metformin may only exert tumor suppressive effects in metabolic phenotypes of high adiposity and metabolic syndrome (28-30). We select to study premenopausal women over postmenopausal women because premenopausal women have a higher baseline breast density which may allow for more notable detection of reduction in breast density (primary study endpoint). Cuzick and colleagues showed that 4.5 years of tamoxifen intervention was associated with a 13% absolute reduction in breast density among women aged 45 years or younger, compared with only 1% reduction among women older than 55 years (31).

The primary study aim is to determine the metformin effect on the risk features of the breast by assessing the modulation effect on breast density. While the specific relationship between breast density and breast cancer has not been established, breast density is one of the strongest and most consistent risk factors for breast cancer. Studies suggest that women with the dense tissue in more than 60-75% of the breast are at 4-6-fold greater risk of developing breast cancer than those with no dense tissue (32-34). In addition, density in more than 50% of the breast could account for about a third of breast cancer (reviewed by (35)). The biological mechanism behind the association between breast density and breast cancer is not completely understood, but breast density may reflect the amount and proliferation of epithelial and stromal cells in the breast and exposure of the breast to mitogens and mutagens (reviewed by (36)). Importantly, breast density is modifiable by exposures that influence breast cancer risk (31, 37-40). We hypothesize that metformin intervention would reduce breast density because metformin has been shown to decrease breast cell proliferation and modulate the IGF axis (7, 8, 26, 27) and both of these have been associated with variation in breast density (41-44). In the proposed project, breast density will be assessed by FWR-MRI, a novel, three-dimensional measure of breast density that will provide more sensitive and quantitative detection of changes in breast density than mammographic measure.

The study will also determine whether the metformin intervention will result in favorable changes in metabolic disturbances and anthropometric measures in the study cohort. Favorable changes in these metabolic disturbances would provide clinical evidence for metformin in risk reduction for breast cancer and other chronic diseases. In addition, we will explore the application of metabolomics as a systems biology approach to assess the chemopreventive mechanisms of metformin and to understand metabolic features affecting systemic and tissue markers of breast cancer risk.

Findings from this study will have wide public health impact because of the growing overweight and obese populations at risk for multiple diseases. With its demonstrated effect in reducing the incidence of diabetes in high risk adults (45), metformin would have a high level of acceptance and uptake in at risk women with high adiposity if it has also been shown to exert favorable activities in breast cancer risk reduction. Importantly, a 10-year cost-effectiveness study showed that metformin is more cost-effective than life-style intervention for diabetes prevention (46). Considering the challenges in maintaining a healthy life style by the majority of general public and the pleiotropic activities of metformin for multiple metabolic disorders, metformin could be developed as an integrated pharmacological approach for at risk women with high adiposity for prevention of multiple diseases.

2.3 Study Endpoints

2.3.1 Breast Density

The primary endpoint is changes in breast density, assessed by MRI-acquired fat-to-water ratio. Measures of mammographic breast density suffer from well recognized limitations that reduce precision, reproducibility, sensitivity and accuracy (47, 48). Mammography also imparts ionization radiation to the breast. Although added risk from repeated mammography is small for the general population (13 per 100,000), it is not insignificant (49), and may be greater for women at risk for breast cancer. Drs. Stopeck and Thompson (Co-Is) have conducted a pilot study of 29 women to determine the application of FWR-MRI to quantitatively measure density in the breast without irradiating the breast or use of contrast agents. The study team has optimized data acquisition and image feature extraction (< 5 min/scan) and demonstrated high reproducibility (r^2=0.997) of FWR-MRI applied to images of the breast. Figure 2 shows example images and FWR-MRI histograms in relation to their corresponding BIRADS categories. Importantly, the study team has selected the fraction of voxels with more water than fat content (Fra50) as the primary measure of breast density derived from FWR-MRI which includes the area of the histogram with < 50% fat. The log(Fra50) measure of breast density produces results that are highly correlated with visualized % density as seen on standard mammography (Spearman rho = 0.946, p < 0.0001, Figure 3). The log(Fra50) is easily derived from FWR-MRI imaging, correlates strongly with mammography density and provides an optimized dynamic range for measuring small changes in density. FWR-MRI for breast density assessment has been implemented at UACC in two NCI-funded ongoing trials led by the Co-Is.

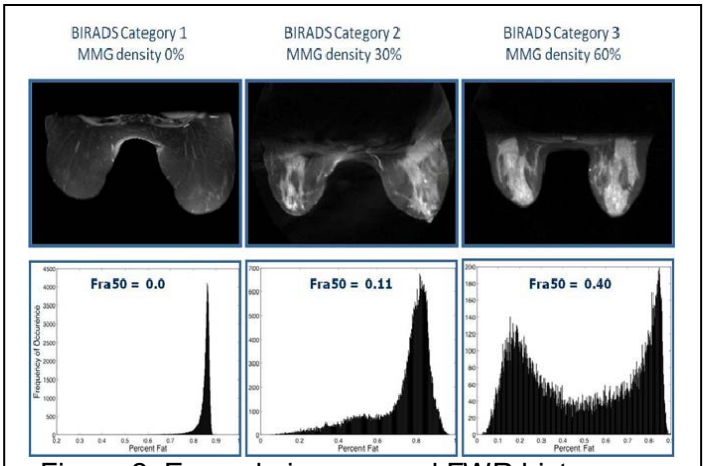

Figure 2. Example images and FWR histograms

Another major strength of FWR-MRI over mammography lies in the number of non-compressed images/segments that can be taken and used in the analysis, yielding a three dimensional image of the breast. This is especially relevant in our proposed cohort in accurate assessment of breast density because a greater compressed breast thickness is expected in overweight and obese women than normal-weight women. FWR-MRI also allows dense areas to be located within the breast rather than seeing a single compressed measurement and can be re-assessed using shorter intervals at no risk or harm to the women on study.

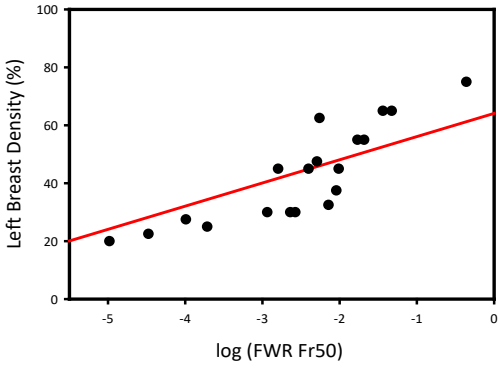

Figure 3. Mammographic density vs. FWR-MRI defined by log(Fra50)

2.3.2 Metabolic Disturbances and Anthropometric Measures

The study will determine the effect of metformin intervention on metabolic disturbances (including measures of circulating levels of insulin, sex steroid hormones, insulin-like growth factor axis, and adipokines) and anthropometric measures.

Insulin. Insulin exerts a mitogenic effect on both normal and neoplastic breast epithelial cells. Hyperinsulinemia and insulin resistance are hypothesized mechanisms underlying the link between obesity and breast cancer risk and prognosis (50). One mechanism by which high levels of insulin might promote breast cancer is by causing a reduction in sex hormone binding globulin production by the liver (51, 52) with a consequent elevation in bioavailable estradiol and stimulation of tumor cell proliferation. Insulin can also induce aromatase activity (53), thus producing a further increase in estrogen biosynthesis. A positive correlation between fasting serum insulin levels and breast cancer risk was observed in several studies (54-56). Metformin has been shown to reduce circulating insulin levels in women with polycystic ovary syndrome (reviewed by (6)) and in non-diabetic breast cancer patients (11).

Sex Steroid Hormones. Breast cancer risk is also partially determined by several hormone-related factors. A large case-control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) showed that premenopausal women with elevated serum levels of testosterone or androstenedione had an increased risk of breast cancer (57). The finding is consistent with observations from two other smaller prospective studies (58, 59). Results on the relationship between breast cancer risk and pre-menopausal blood levels of estradiol are less consistent (57, 59-61). Metformin has been shown to decrease serum testosterone and androstenedione levels (9, 10) and increase serum sex steroid binding globulin (9, 10) in women with polycystic ovary syndrome.

Insulin-like Growth Factor Axis. Insulin-like growth factors 1 and 2 are among the most potent stimulators of cell proliferation and differentiation in humans. Their bioavailability is regulated by a family of IGF binding proteins (IGF-BP), which can bind and inactivate IGFs but also increase their activity under specific conditions. IGF-BP3 binds approximately 75-90% of circulating IGF-1 and IGF-2 and may be the most important determinant of IGF bioavailability (62). These systemic hormones are expressed in most tissues including the breast and are locally acting growth factors with paracrine and autocrine functions. Epidemiological evidence suggests that elevated levels of serum IGF-1 or relative ratios of IGF-1 to IGF-BP3 may be associated with an increased risk of breast cancer in premenopausal women (63-65). Metformin has been shown to increase IGF-BP1 but have no effect on IGF-1 in women with polycystic ovary syndrome (7, 8).

Adipokines. Leptin and adiponectin are adipokines produced predominantly or exclusively by adipocytes of white adipose tissue. A number of studies have shown that low serum adiponectin levels and high serum leptin levels are associated with an increased risk for breast cancer (66-70), although some studies found no association between circulating leptin level and breast cancer risk (71-73). Nevertheless, studies have shown that leptin measurement is more sensitive to a number of variables related to specimen collection (74, 75). The circulating leptin concentration is directly proportional to the degree of adiposity (76). In addition, serum leptin levels are 2-3 times higher in women than in men after adjusted for age and BMI (77). Leptin is a mitogen for various cell types including normal and transformed breast epithelial cells (78, 79). Leptin has been shown to induce proliferation (80-82), increase the expression of proteolytic enzymes that are essential in metastatic process (83), and stimulate angiogenesis (84, 85). In addition, leptin interferes with insulin signaling, and insulin resistance syndrome is accompanied by hyperleptinaemia as well as hyperinsulinaemia, which allows for excessive endocrine activity of these proteins at target sites (86-88). In contrast to leptin, there is an inverse relationship between the plasma adiponectin concentration and body fat mass (89, 90). Its production in adipose tissue is suppressed in the insulin resistance syndrome and obesity with consequent hypoadiponectinaemia (91). Adiponectin has been shown to suppress proliferation of human breast cancer cells (82, 92, 93). It was also reported to suppress angiogenesis by a process which involves activation of members of the caspase

group of apoptotic factors (94). In diabetics, metformin treatment resulted in favorable changes in circulating levels of leptin and adiponectin (12, 13).

Anthropometric Measures. Studies suggested that weight reduction reduces risk of postmenopausal breast cancer. For example, in the Iowa Women's Health Study, women who lost >5% of their body weight before menopause had 40% fewer breast cancers in the postmenopausal period compared with women who continued to gain weight (95). In addition to weight gain, body fat distribution has also been suggested to play a role in the risk of breast cancer. Positive association between central adiposity and breast cancer risk was observed in some studies (96-99). Studies have shown that metformin intervention resulted in weight reduction and decreased waist circumference in overweight or obese individuals without type 2 diabetes, although these trials were small and of weak design (reviewed by (14)).

2.3.3 Metabolomics in NAF and plasma

The study will explore the application of metabolomics as a systems biology approach to assess the chemopreventive mechanisms of metformin. The global profiles of biochemicals and metabolites in nipple aspirate fluid and plasma will be assessed through unbiased metabolome wide analysis to determine the metabolic changes induced by metformin in comparison with placebo. We will also explore whether metformin-induced metabolic changes correlate with metformin-induced changes in markers of breast cancer risk or side effects. In a cross-sectional fashion, we will explore the correlations between baseline metabolic features with breast density and circulating levels of hormones and cytokines.

Metabolomics is a comprehensive analysis of metabolite levels in whole organisms and the investigation of how stimuli such as diet, lifestyle, environment, genetic effects, and pharmaceutical interventions influence the metabolite levels (100). Metformin has demonstrated diverse biological activities in different cells/tissues. Application of metabolomics to this project would represent a systems biology approach to understand the mechanisms for metformin-associated changes in markers of breast cancer risk or adverse effects in healthy overweight/obese women. A recent pilot metabolomics study in 15 type 2 diabetic patients showed that treatment with metformin for 3 months resulted in changes in a number of endogenous metabolites (101). In addition to its effects on glucose and lipoprotein metabolism pathways, metformin also modulated bacteria and nutrition metabolism (101).

For the proposed project, we also plan to explore the metabolomic profiles in NAF. NAF contain breast epithelial cells as well as proteins and other cellular metabolites secreted from the ductal epithelium. It can be collected noninvasively, inexpensively, and repeatedly with minimal or no discomfort. We have had extensive experience in NAF collection and determination of biomarkers in NAF in early phase clinical trials. We have shown a reduction in prostaglandin E_2 levels in NAF following a 6-week intervention of sulindac in women at increased breast cancer risk (102). In our recently completed study, we found that menopausal status significantly affected expression of certain proteins in NAF but the effect is less notable in plasma (see Table 1), suggesting that NAF biomarkers may provide information about the breast microenvironment that might not be reflected in plasma.

Table 1. NAF and plasma biomarker expression differs by menopausal status.

NAF	EGF (ng/g PT)	TGF-β1 (ng/g PT)	Adiponectin (µg/g PT)
Pre-menopausal	7,759±2,854	2,079±3,027	11.6±9.1
Post-menopausal	2,532±4,354	361±357	26.3±28.5
P-value	0.0022	<0.0001	0.0880
Plasma	EGF (pg/ml)	TGF-β1 (ng/ml)	Adiponectin (µg/ml)
Pre-menopausal	34.1±29.0	9.92±3.77	9.67±4.19
Post-menopausal	21.6±13.5	10.47±3.13	13.37±6.14
P-value	0.2891	0.5097	0.0607

2.3.4 Tissue Biomarkers

The study will also explore the effect of metformin intervention on tissue biomarkers in breast tissue collected in a subgroup of participants. The collection of pre- and post-treatment tissue biopsy in a subset of subjects will allow us to assess changes in tissue architecture as well as to measure change in metformin molecular and metabolic targets in normal breast tissue. In addition, tissue level information will allow us to conduct correlative studies with our primary endpoint (change in breast density) and secondary endpoints (change in metabolites in NAF). These analyses will provide much needed insight on the potential mechanisms of metformin action at the tissue level and aid in validating FWR-MRI and NAF as surrogate measures for tissue changes with metformin intervention.

The ongoing sulindac/AI trial (IRB#12-0080-04) led by Drs. Stopeck and Thompson has established the infrastructure for performing ultrasound-guided core needle biopsy for clinical research. Currently, 100% of enrolled participants have consented to undergo the optional core needle biopsy procedures and have found the procedure acceptable and relatively pain-free. All have tentatively agreed to undergo a second procedure for breast tissue procurement as well. We plan to adapt the experience gained from the sulindac/AI trial to facilitate the collection of breast tissue in the metformin study. We conservatively estimate that 50% of women will consent to provide core biopsy specimens.

3. SUMMARY OF STUDY PLAN

This is a Phase II double-blind, randomized, placebo-controlled trial of metformin to determine its potential effects on recognized and putative markers of breast cancer risk in overweight or obese premenopausal women who have metabolic syndrome. The study endpoints are modulation of breast density and metabolic disturbances, risk factors associated with breast cancer. In addition, the study will explore the application of metabolomics as a systems biology approach to assess the chemopreventive mechanisms of metformin and to understand factors affecting markers of breast cancer risk. The study will also explore the effect of metformin intervention on tissue architecture as well as cellular and molecular targets in breast tissue collected in a subgroup of participants.

At the consenting/screening visit, participants will sign informed consent and undergo the following procedures for screening: collection of medical history and medication usage history, demographic information (age, race/ethnicity), anthropometric measurements (weight (wt), height, waist circumference, waist-hip ratio), breast cancer risk information (family and personal history of breast cancer, age at menarche, parity, and prior breast biopsy), information on menstrual patterns/cycles, and a fasting blood sample for complete blood count with differential (CBC/w diff) and comprehensive metabolic panel (CMP).

Participants who meet all selection criteria will return in the midluteal phase of the menstrual cycle, when feasible, to undergo baseline evaluation including collection of a fasting blood for research biomarkers, collection of NAF for metabolomic analysis, MRI assessment of breast density, collection of wt, waist circumference, and waist-hip ratio measurements, and the optional core needle biopsy for breast tissue collection. Women who cannot fit into the MRI scanner due to the large body size will continue the study and undergo all other study procedures. Participants will also complete the Arizona Food Frequency Questionnaire (AFFQ) to measure usual dietary intake and the Arizona Activity Frequency Questionnaire (AAFQ) to assess usual physical activity.

Following completion of baseline evaluation, participants will be randomized to receive metformin or placebo for 12 months. We plan to randomize 150 eligible participants (75 per arm). With an anticipated attrition rate of 20% or less, we expect to have at least 60 women per

arm completing the intervention. Participants will be asked to take one 850 mg metformin or one placebo tablet daily with a meal for the first four weeks, then two 850 mg metformin or two placebo tablets daily with meals for the remainder of the study. The same starting dose and gradual dose escalation up to 2,000 mg per day is used to minimize gastrointestinal symptoms for glycemic control in diabetics (GLUCOPHAGE® prescribing information).

Participants will be asked to keep an adverse event diary and menstrual calendar throughout the study. In addition, participants will be provided with an intake calendar for recording medication usage. Study personnel will contact study participants within a week after study agent has been initiated and within a week following scheduled dose increase to assess compliance and any potential problems. Participants will return to the clinic at months 3, 6, and 9 after initiation of agent intervention to undergo safety and compliance evaluation. For the month 6 visit, participants will be scheduled to return to the clinic in the midluteal phase of the menstrual cycle, when feasible. In addition to undergoing study procedures described above, participants will undergo wt, waist circumference, and waist-hip ratio measurements, have a fasting blood and NAF collected for research biomarkers, undergo MRI assessment of the breast if MRI was performed at the baseline visit, return unused pills and receive a new supply of study medication, and undergo the optional core needle biopsy. Study personnel will contact study participants at least once and as needed between clinic visits to assess compliance and any potential problems.

At the end of the 12-month agent intervention, participants will return to the clinic in the midluteal phase of the menstrual cycle, when feasible, to collect wt, waist circumference, and waist-hip ratio measurements, return unused drugs, review side effects, and have a fasting blood sample collected for safety labs and research biomarkers. Participant will also undergo NAF collection for metabolomics, and MRI assessment of the breast if MRI was performed at the baseline visit. Participants will also complete the AFFQ and AAFQ to assess any changes in dietary intake and physical activity during the intervention. Following the study intervention, participants will be followed for 2 weeks for any adverse reactions.

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria

- Premenopausal women
- 21-54 years of age
- No change in menstrual patterns for the past 6 months preceding the time of registration
- Have a BMI of 25 kg/m^2 or greater
- Large waist circumference
    - ≥ 88 cm (≥35 inches) or
    - ≥ 80 cm (≥31 inches) for Asian Americans, individuals with polycystic ovary syndrome, or individuals with non-alcoholic fatty liver disease
- Have at least one other component of metabolic syndrome (103) reported below:
    - Elevated triglycerides (≥ 150 mg/dL (1.7 mmol/L)) or on drug treatment for elevated triglycerides
    - Reduced HDL-C (< 50 mg/dL (1.3 mmol/L)) or on drug treatment for reduced HDL-C
    - Elevated blood pressure (≥ 130 mm Hg systolic blood pressure or ≥85 mm Hg diastolic blood pressure) or on antihypertensive drug treatment in a patient with a history of hypertension
    - Elevated fasting glucose (≥100 mg/dL)

- Mammogram negative for breast cancer within the 12 months preceding the time of registration for women ≥ 50 years of age
- Ability to understand and the willingness to sign a written informed consent document

4.2 Exclusion Criteria

- Postmenopausal women
    - Amenorrhea for at least 12 months (preceding the time of registration), or
    - History of hysterectomy and bilateral salpingo-oophorectomy, or
    - At least 55 years of age with prior hysterectomy with or without oophorectomy, or
    - Age 35 to 54 with a prior hysterectomy without oophorectomy OR with a status of ovaries unknown with documented follicle-stimulating hormone level demonstrating elevation in postmenopausal range
- Women who are pregnant, planning pregnancy within the next year, or lactating/breastfeeding
- On treatment with any drug for diabetes
- Have uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or any illness that would limit compliance with study requirements
- Have received chemotherapy and/or radiation for any malignancy (excluding non-melanoma skin cancer and cancers confined to organs with removal as only treatment) in the past 5 years (preceding the time of registration)
- Have received other investigational agents within the past 3 months (preceding the time of registration)
- Have a history of lactic acidosis or risk factors for lactic acidosis
- Have renal disease or dysfunction (creatinine ≥ 1.4 mg/dL)
- Have hepatic dysfunction (bilirubin > 1.5 x ULN unless with Gilberts syndrome or AST/ALT > 3 x ULN)
- Have a history of alcoholism or high alcohol consumption (average of > 3 standard drinks/day)
- Have a history of allergic reactions to metformin or similar drugs
- Have a history of severe claustrophobia
- Have electrically, magnetically, or mechanically activated implants including cardiac pacemaker, cochlear implants, magnetic surgical clips or prostheses
- Have breast implants

5. AGENT ADMINISTRATION

5.1 Dose Regimen and Dose Groups

- Subjects will be randomly assigned to the following treatment groups:
    - Metformin, 850 mg tablets
    - Placebo tablets
- Duration of treatment is 12 months (52 $\pm$ 4 wks).

5.2 Study Agent Administration

- For the first four weeks, 1 metformin or placebo tablet will be taken daily.
- For the remaining treatment period, 2 metformin or placebo tablets will be taken daily. Depending on the tolerability, 2 tablets may be taken together with a meal or taken separately (i.e., one tablet twice a day).

- Tablets should be taken with food, preferably with the evening meal.
- Tablets should be swallowed with a full 8 oz glass of water.

5.3. Contraindications

Metformin is contraindicated in patients with

- Renal disease or renal dysfunction
- Known hypersensitivity to metformin hydrochloride
- Acute or chronic metabolic acidosis
- Hepatic impairment, as defined in the exclusion criteria

Because use of iodinated contrast materials may result in acute alteration of renal function and have been associated with lactic acidosis in patients receiving metformin, patients who plan to undergo radiologic studies involving intravascular administration of iodinated contrast materials will not be permitted to begin study medication until at least 48 hours following the procedure and only after renal function has been re-evaluated and found to be normal (serum creatinine < 1.4 mg/dL).

5.4 Concomitant Medications

Participants may not use non-study metformin or other biguanides while on study.

Cationic drugs (e.g., amiloride, digoxin, morphine, procainamide, quinidine, quinine, cimetidine, ranitidine, triamterene, trimethoprim, or vancomycin) have the potential for interaction with metformin by competing for common renal tubular transport systems which may interfere with the disposition of metformin. Although such interactions remain theoretical (except for cimetidine), careful patient monitoring is recommended in patients who are taking cationic medications that are excreted *via* the proximal renal tubular secretory system.

All medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs taken by the participant will be documented on the concomitant medication CRF and will include: 1) start and stop date, dose and route of administration, and indication. Medications taken for a procedure (e.g., biopsy) should also be included.

If a subject requires a radiologic exam involving intravascular administration of iodinated contrast materials while participating in the study, she must discontinue study agent for at least 48 hours prior to having the exam and will not be permitted to restart study medication until at least 48 hours following the procedure and only after renal function has been re-evaluated and found to be normal (serum creatinine < 1.4 mg/dL).

5.5 Dose Modification

The NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4 will be used for toxicity reporting. For grade 1 adverse events or less, no dose modification will be made.

For grade 2 adverse events probably or possibly related to study agent persisting for more than three days during the first 4 weeks of dosing with 1 tablet of 850 mg metformin or placebo daily, study drug will be held. Study drug will be reintroduced at the same dose when the adverse event resolves to Grade 1 or below within 2 weeks. If the Grade 2 event does not resolve to Grade 1 or below within 2 weeks, participants will be removed from the study and followed for resolution of the adverse event.

For grade 2 adverse events probably or possibly related to study agent persisting for more than three days after taking 2 tablets of 850 mg metformin or placebo daily, study drug will

be held. Study drug will be reintroduced at 50% dose (1 tablet of 850 mg metformin or placebo daily) when the adverse event resolves to Grade 1 or below within 2 weeks. If the Grade 2 event does not resolve to Grade 1 or below within 2 weeks, participants will be removed from the study and followed for resolution of the adverse event. After reinstitution of 50% dose (1 tablet of 850 mg metformin or placebo daily) for a minimum of 2 weeks, the full dose (2 tablets of 850 mg metformin or placebo daily) may be reintroduced at the discretion of the study physician. If the Grade 2 adverse event recurs and persists for more than three days following reinstitution of the full dose and is considered definitely, probably or possibly related to study agent, the study agent will be held and reintroduced at 50% dose (1 tablet of 850 mg metformin or placebo daily) if the adverse event resolves to Grade 1 or below within 2 weeks. The dose will then be kept at the reduced dose for the duration of the study. If the Grade 2 event does not resolve to Grade 1 or below within 2 weeks, participants will be removed from the study and followed for resolution of the adverse event. If participants do not wish to be re-challenged with the full dose, they may remain on study at 50% dose.

For Grade 2 or Grade 3 adverse events not considered related to study agent, the study agent may be stopped and resumed at the discretion of study physician.

Grade 3 events possibly, probably, and definitely related to study agent, and Grade 4 events regardless of relationship to study agent, will result in permanent withdrawal of study agent. Subjects will be followed for resolution of adverse events. The Principal Investigator and Medical Director will be notified immediately of any grade 3 or 4 adverse events. The study investigators will decide if the adverse event is related to the agent.

All AEs, including lab abnormalities will be followed for 2 weeks or longer for resolution according to good medical practices and documented as such.

6. PHARMACEUTICAL INFORMATION

6.1 Metformin

Metformin is a biguanide indicated as an adjunct to diet and exercise to improve glycemic control in adults and children with type 2 diabetes mellitus. Metformin is one of the most widely used oral antihyperglycemic drugs in the management of type 2 diabetes. It has been used worldwide for over 40 years to treat type 2 diabetes, and was approved in the United States (US) by the Food and Drug Administration (FDA) as an antidiabetic in 1995 (104). It is also used off-label for polycystic ovary syndrome and metabolic syndrome. The major mechanism of metformin action *in vivo* involves suppression of hepatic gluconeogenesis and glucose output, which is associated with a decline in circulating glucose concentration and a secondary decline in insulin levels (105).

Beneficial effects have also been observed in non-diabetics, including treatment of weight gain induced by antipsychotic medications (106), polycystic ovary syndrome (6), nonalcoholic fatty liver (107) and diabetes prevention in high-risk subjects (108). It has a demonstrated safety profile and good tolerability in both diabetics and non-diabetics.

6.2 Reported Adverse Events and Potential Risks

Metformin is considered to have a favorable safety profile in diabetes patients (109). In a US double-blind clinical trial for patients with type 2 diabetes (metformin n=141; placebo n=145), gastrointestinal symptoms such as diarrhea (53.2% *vs*. 11.7% placebo), nausea and vomiting (25.5% *vs*. 8.3% placebo), abdominal discomfort (6.4% *vs*. 4.8% placebo), flatulence (12.1% *vs*. 5.5% placebo), indigestion (7.1% *vs*. 4.1% placebo), and asthenia (9.2% *vs*. 5.5% placebo) were the most common reactions to the drug. These symptoms were dose-dependent,

transient, and resolved spontaneously with continued use (Glucophage® (metformin hydrochloride tablets) label information). Diarrhea led to discontinuation of medication in 6% of subjects treated with metformin hydrochloride. Additional adverse reactions were reported in 1% to 5% of subjects receiving active drug; those more commonly reported than in the placebo group include abnormal stools, hypoglycemia, myalgia, lightheaded, dyspnea, nail disorder, rash, increased sweating, taste disorder, chest discomfort, chills, flu syndrome, flushing and palpitation (Glucophage® (metformin hydrochloride tablets) label information).

A recently published multicenter, randomized, placebo-controlled trial in patients with type 2 diabetes reported on vitamin B_{12} deficiency with long-term metformin treatment. Compared with placebo, metformin treatment was associated with a mean decrease in vitamin B_{12} concentration of ~19% (95% confidence interval (CI) –24% to –14%; p<0.001). The absolute risk of vitamin B_{12} deficiency (<150 pM) at study end was 7.2 percentage points higher in the metformin group than in the placebo group (95% CI 2.3 to 12.1; p=0.004) (110).

According to the package insert, lactic acidosis is a rare, but serious, metabolic complication that can occur due to metformin accumulation during treatment with metformin hydrochloride. When it occurs, it is fatal in approximately 50% of the cases. Lactic acidosis may also occur in association with a number of pathophysiologic conditions, including diabetes mellitus and whenever there is significant tissue hypoperfusion and hypoxemia. Lactic acidosis is characterized by elevated blood lactate levels (>5 mmol/L), decreased blood pH, electrolyte disturbances with an increased anion gap, and an increased lactate/pyruvate ratio. Metformin plasma levels >5 µg/mL are generally found when metformin is implicated as the cause of lactic acidosis.

Reported incidence of lactic acidosis in patients receiving metformin hydrochloride is very low (approximately 0.03 cases/1000 patient-years, with approximately 0.015 fatal cases/1000 patient-years). In more than 20,000 patient-years of exposure to metformin in clinical trials, there were no reports of lactic acidosis. Reported cases have occurred primarily in diabetic patients with significant renal insufficiency, including both intrinsic renal disease and renal hypoperfusion, and in those with multiple concomitant medical/surgical problems and who take multiple concomitant medications. Patients with congestive heart failure require pharmacologic management, in particular those with unstable or acute congestive heart failure who are at risk of hypoperfusion and hypoxemia and increased risk of lactic acidosis (Glucophage® (metformin hydrochloride tablets) label information.

The risk of lactic acidosis increases with the degree of renal dysfunction and patient age. Drug should be withheld in the presence of any condition associated with hypoxemia, dehydration, or sepsis. Because impaired hepatic function can significantly impair the ability to clear lactate, metformin should generally be avoided in patients with clinical or laboratory evidence of hepatic disease. Alcohol can potentiate the effects of metformin hydrochloride on lactate metabolism. Drug treatment should be temporarily discontinued prior to any intravascular radiocontrast study and for any surgical procedure (111). The onset of lactic acidosis is often subtle, and accompanied by nonspecific symptoms such as malaise, myalgia, respiratory distress, increasing somnolence, and nonspecific abdominal distress. There may be associated hypothermia, hypotension, and resistant bradyarrhythmias with more marked acidosis (Glucophage® (metformin hydrochloride tablets) label information).

In support of the previously reported low risk of lactic acidosis, a recently published systematic review assessed the risk with metformin use in type 2 diabetes mellitus. Pooled data from 347 comparative trials and cohort studies revealed no cases of lactic acidosis in 70,490 patient-years of metformin use. The authors concluded that there was no evidence from prospective comparative trials or from observational cohort studies that metformin is associated

with an increased risk of lactic acidosis compared to other antihyperglycemic treatments (112, 113).

Unlike sulfonylureas, metformin does not produce hypoglycemia in either type 2 diabetes patients or normal subjects and does not cause hyperinsulinemia (109).

Carcinogenicity studies have been performed in rats (dosing duration of 104 weeks) and mice (dosing duration of 91 weeks) at doses up to and including 900 mg/kg/day and 1500 mg/kg/day, respectively. Both doses are approximately four times the maximum recommended human daily dose of 2000 mg based on body surface area. No evidence of carcinogenicity was found in either male or female mice or in male rats. There was, however, an increased incidence of benign stromal uterine polyps in female rats at 900 mg/kg/day (109). Metformin was not mutagenic in the Ames, mouse lymphoma, human lymphocyte chromosome aberration, or mouse micronucleus tests (109).

The fertility of male and female rats was unaffected by metformin when administered at doses as high as 600 mg/kg/day, which is approximately three times the recommended human daily dose based on body surface area (109).

6.3 Source of Study Agent

Metformin tablets, 850 mg will be purchased from a commercial source through the University of Arizona Cancer Center (UACC) Research Pharmacy. We will contract with Pharm Ops, Inc (Phillipsburg, NJ) to manufacture a clinical supply of the placebo tablets using inert excipients. Pharm Ops, Inc. has manufactured placebo products for a number of NCI-sponsored chemoprevention trials.

6.4 Agent Storage and Distribution

Metformin and placebo tablets will be stored in the UACC Research Pharmacy at room temperature [20-25°C] and protected from environmental extreme. It will be dispensed to the study staff in light-resistant containers for distribution to the study participants.

6.5 Registration/Randomization

Subjects will be considered registered on the date they sign the approved informed consent document with a member of the study staff. Randomization will be performed using computer-generated random permuted blocks.

6.6 Blinding and Unblinding Methods

To retain the blind, metformin and placebo tablets will not be identified by product names but by a unique randomization number. The randomization number is assigned to the subject upon completion of eligibility evaluation.

A list of randomization numbers linked to active or placebo agent will be generated by the study statistician or the designated data manager and forwarded to the UACC Research Pharmacy. The UACC Research Pharmacy will dispense the study agent to the study staff by randomization number. The study agent bottles will be identified with the subject randomization number but with no product information on the label. The study staff will dispense the product to subjects based on the assigned randomization number. None of the staff interacting with subjects will know the link between randomization number and actual product. The code that identifies the product will be kept by the study statistician or the designated data manager.

Unblinding is not expected to occur until all subjects complete the intervention and data entry is complete. If deemed medically necessary, study agents may be unblinded by the

Principle Investigator in consultation with the Medical Director in the event of a serious adverse event.

6.8 Agent Disposal

Unused agents will be returned to the clinic for compliance check and then be disposed according to the UA waste disposal guidelines.

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

Study Events	Consenting/ Screening	Baseline/ Randomization	Intervention (12 month (52 ± 4 wks))								
			Months 1 - 3	Month 3 Visit (13 ± 2 wks)	Months 4 - 6	Month 6 Visit (26 ± 4 wks)	Months 7 - 9	Month 9 Visit (39 ± 2 wks)	Months 10 - 12	Month 12 Visit (52 ± 4 wks)	Follow up
Consent, med records release form	x										
Medical history, performance status	x										
anthropometric measurements	x	x				x				x	
Vital signs (Temp, BP, Pulse)	x	x		x		x		x		x	
Concomitant meds	x	x		x		x		x		x	
Breast cancer risk assessment	x										
Menstrual pattern/cycle review	x	x		x		x		x		x	
Menstrual cycle diary	x	x	x	x	x	x	x	x	x	x	
Fasting blood (CBC/Diff, CMP, lipids, FSH[2],estradiol[2])	x									x	
Fasting blood for research endpoints		x				x				x	
Urine pregnancy test	x	x		x		x		x		x	
Urine for research endpoints		x				x				x	
AFFQ, AAFQ		x								x	
NAF collection		x				x				x	
Breast MRI[3]		x				x				x	
Core biopsy, if consented		x				x					
Final eligibility assessment		x									
Randomization		x									
Dispense study agent		x				x					
Intake calendar			x	x	x	x	x	x	x	x	
Adverse events diary		x	x	x	x	x	x	x	x	x	x
Adverse events review		x		x		x		x		x	x
Return study agent						x				x	
Compliance assessment				x		x		x		x	
Case report form completion	x	x		x		x		x		x	x
Telephone/email contact[1]			x		x		x		x		

[1] Study personnel will contact subjects within a week after study agent has been initiated and within a week following scheduled dose increase to assess compliance and any potential problems. Additional periodic telephone or email contact will occur between study visits and as needed to review study procedures, adverse events, concomitant medications, and to address any subject concerns.

[2] FSH (follicle-stimulating hormone) and/or estradiol at screening for women with uncertain menopausal status.

[3] Women who cannot fit into the MRI scanner due to the large body size will continue the study and undergo all other study procedures.

7.2 Consenting/Screening

Potential subjects will present to clinic for a detailed discussion of the protocol with the study coordinator. Signed informed consent will be obtained prior to any study-related activities or procedures being conducted. Subjects are registered onto the protocol on the day of consent. Participants will then undergo the following procedures for screening.

- Review of medical history and medication usage history
- Collection of demographic information (age, race/ethnicity).
- Collection of anthropometric measurements (weight (wt), height, waist circumference, waist-hip ratio). Body weight will be measured with participants standing on a calibrated scale with minimal movement with hands by their side and shoes and excess clothing removed. Waist and hip circumference will be measured in a standing position with a flexible tape. Waist circumference will be measured at the midpoint between the lowest rib and the top of the iliac crest on the bare abdomen. Participants will be asked to relax and exhale for the measurement. Hip circumference will be measured at the level of the greatest protrusion of the gluteal muscles, with minimal clothing. Participants will be asked to stand with their weight evenly distributed on both feet and legs slightly parted, making sure not tense the gluteal muscles. When recording the measurement, the study personnel will ensure that the tape is not too tight or too loose, is lying flat on the skin, and is horizontal.
- Collection of breast cancer risk information (family and personal history of breast cancer, age at menarche, parity, and prior breast biopsy).
- Collection of information on menstrual patterns/cycles.
- Measures of vital signs (temperature, blood pressure and pulse).
- A fasting blood sample for complete blood count with differential (CBC/w diff), comprehensive metabolic panel (CMP), lipids, and follicle-stimulating hormone (FSH) and/or estradiol for women with uncertain menopausal status. The screening blood work may be repeated, if clinically indicated.
- Urine pregnancy test.

7.3 Evaluations During Study Intervention

Participants who meet all selection criteria will undergo the following baseline procedures. When feasible, these procedures will be scheduled in the midluteal phase of the menstrual cycle.

- Anthropometric measurements including wt, waist circumference, waist-hip ratio.
- A fasting blood for research biomarkers.
- Collection of urine for urine pregnancy test and research tests.
- Vital signs.
- Update information on menstrual patterns/cycles.
- Update medication usage.
- Completion of the Arizona Food Frequency Questionnaire (AFFQ) to measure usual dietary intake and the Arizona Activity Frequency Questionnaire (AAFQ) to assess usual physical activity.
- Nipple aspirate fluid, for those who are able to produce NAF, for metabolomic analysis. Breasts will be warmed and massaged. Nipple fluid will be aspirated by a hand-held aspirator.

- MRI assessment of breast density. All MRI images will be acquired on a 3T MRI scanner located in the department of Radiology of the University of Arizona Medical center. Fat-water imaging will be performed using the radial Gradient-Echo and Spin-Echo (GRASE) technique developed by co-investigator, Dr. Altbach (114). Women who cannot fit into the MRI scanner due to the large body size will continue the study and undergo all other study procedures.
- Optional core needle biopsy. For participants who consent to this optional procedure, the medical specialist will use a 14-gauge needle under the ultrasound guidance to obtain up to 8 tissue cores from one of the breasts.

Following completion of baseline evaluation, participants will be randomized to receive metformin or placebo for 12 months. Participants will receive a supply of study medication. Participants will be asked to take one 850 mg metformin or one placebo tablet daily with a full 8 oz glass of water with a meal for the first four weeks, then two 850 mg metformin or two placebo tablets daily with meals for the remainder of the study. The same starting dose and gradual dose escalation up to 2,000 mg per day is used to minimize gastrointestinal symptoms for glycemic control in diabetics (GLUCOPHAGE® prescribing information).

Participants will be asked to keep an adverse event diary and menstrual calendar throughout the study. In addition, participants will be provided with an intake calendar for recording medication usage. Study personnel will contact study participants within a week after study agent has been initiated and within a week following scheduled dose increase to assess compliance and any potential problems.

Participants will return to the clinic at month 3 (13 ± 2 wks) to undergo the following procedures:

- Collection of urine for urine pregnancy test.
- Vital signs.
- Update information on menstrual cycles.
- Update medication usage.
- Side effect evaluation.

Participants will return at month 6 (26 ± 4 wks) to undergo the following procedures:

- Collection of urine for urine pregnancy test and research tests.
- Vital signs.
- Update information on menstrual patterns/cycles.
- Update medication usage.
- Side effect evaluation.
- Return unused pills.
- Receive a new supply of study medication.
- Collection of wt, waist circumference, waist-hip ratio measurements.
- Collection of a fasting blood and NAF sample for research biomarkers.
- MRI assessment of the breast. Women who cannot fit into the MRI scanner due to the large body size will not undergo the MRI assessment.
- Optional breast core biopsy.

When feasible, the month 6 procedures will be scheduled in the midluteal phase of the menstrual cycle.

Participants will return to the clinic at month 9 (39 ± 2 wks) after initiation of agent intervention to undergo the following procedures:

- Collection of urine for urine pregnancy test.
- Vital signs.
- Update information on menstrual patterns/cycles.
- Update medication usage.
- Side effect evaluation.

Study personnel will contact study participants at least once and as needed between clinic visits to assess compliance and any potential problems.

7.4 Evaluations at Completion of Study Intervention

At the end of the 12-month agent intervention (52 ± 4 wks), participants will return to the clinic to undergo the following procedures:

- Collection of urine for urine pregnancy test and research tests.
- Vital signs.
- Update information on menstrual patterns/cycles.
- Update medication usage.
- Return unused drugs.
- Side effects evaluation.
- Anthropometric measurements including wt, waist circumference, waist-hip ratio.
- A fasting blood for CBC/CMP/lipids and research biomarkers.
- NAF collection.
- MRI assessment of breast density. Women who cannot fit into the MRI scanner due to the large body size will not undergo the MRI assessment.
- Completion of AFFQ and AAFQ.

When feasible, the month 12 procedures will be scheduled in the midluteal phase of the menstrual cycle.

Participants will be instructed to take the study agent until the day of the last study procedure.

7.5 Post-intervention Follow-up Period

Following the study intervention, participants will be followed for 2 weeks for any adverse reactions.

8. SPECIMEN MANAGEMENT

8.1 Collection and Handling Procedures

Blood Samples

Clinical labs

Fasting blood samples will be collected during screening and at the end of intervention for CBC with differentials, and CMP/lipids. FSH and/or estradiol will be done at screening for women with unknown menopausal status. Approximately 15 ml of blood will be drawn to one 3-5 ml EDTA and one 7-10 ml SST or tiger top Vacutainer tubes as directed by the clinical lab, and labeled with the subject name. Samples will be processed and stored according the standard

protocol for each. The serum tube (SST or tiger top for CMP) will be allowed to clot for approximately 30 minutes at room temperature then centrifuged within 1 hour of collection for serum separation. The EDTA tube (for CBC/diff) will be gently inverted to mix for anticoagulation. All samples will be stored under refrigeration prior to transfer to the commercial laboratory facility on the same day as obtained with a completed lab requisition.

Research biomarkers

Fasting blood samples will also be collected during baseline, month 6 and at the end of intervention for research biomarker analyses. Approximately 30 ml of fasting blood will be drawn into two 10 ml SST or tiger top Vacutainer tubes and one 10 ml heparin Vacutainer tube. The blood in the SST or tiger top tubes will be allowed to clot for approximately 30 minutes at room temperature then centrifuged for serum separation. The heparin tube will be gently inverted to mix for anticoagulation. Serum from the SST tubes will be aliquoted into 10 x 1-2 ml cryovials and plasma from the heparin tube will be aliquoted into 5 x 1-2 ml cryovials. Samples will be labeled with the study ID, subject ID, and date of collection and stored at -80°C until analyses.

NAF will be collected at baseline, month 6 and at the end of intervention. NAF will be collected in small capillary tubes. The capillary tubes will then be stored in a 5 ml cyrotube. Cryotubes will be labeled with the study ID, subject ID, and date of collection and stored at -80°C until analyses.

Clean catch urine will be collected at baseline, month 6 and at the end of intervention. Urine will be aliquoted into 4 x 1-2 ml cryovials. Cryovials will be labeled with the study ID, subject ID, and date of collection and stored at -80°C until analyses.

Breast Biopsies

Optional breast biopsies will be performed at baseline and month 6. Up to eight tissue cores will be collected at each procedure. Half of the tissue will be immediately fixed in 10% neutral buffered formalin for 24 hours, and then transferred to 70% ethanol prior to routine processing and paraffin embedding (FFPE). Representative FFPE specimens will be reviewed by UAMC pathology department. The remaining half will be placed in cryogenic vials or bags and flash frozen in liquid nitrogen and then stored at -80°C for future studies. Samples will be labeled with the study ID, subject ID, and date of collection.

8.2 Shipping instructions

Research specimens will be hand delivered to Dr. Sherry Chow's laboratory for final storage and analyses. Serum/plasma and frozen tissue will be delivered on dry ice. Paraffin-embedded tissue blocks will be delivered at room temperature. Each delivery is to be accompanied with a sample list which is to be signed by the senders and recipients. The recipients will be instructed to return a copy of the signed receipt to the senders. The delivery address is listed below.

Catherine Cordova c/o Chow Laboratory
Arizona Cancer Center, Room 4971
1515 N. Campbell Ave.
Tucson, AZ 85724
Phone: (520) 626-5433
ccordova@uacc.arizona.edu

8.3 Tissue Banking

Biological specimens collected for this study that are not expended in analyses described in this protocol may be retained ("banked") for future research testing only with the

consent of the subject. With that consent, remaining serum/plasma and frozen tissue will be stored at -80°C for future testing; paraffin embedded tissue will be stored in locked cabinets at room temperature. A log will be kept for all stored specimens regarding the date of collection, subjection identification number, study number, and the storage location.

9. STATISTICAL CONSIDERATIONS

9.1 Analysis Plan

The primary study endpoint is to compare the change in breast density as measured by FWR-MRI (when measured at baseline, 6 and 12 months) between metformin and placebo groups. Additional endpoints such as systemic hormone and cytokines, body weight, waist circumference, and waist-hip ratio will be measured at baseline, 6 and 12 months. Analysis for all study endpoints will be based on a linear mixed-effects model for the observed values across time, to adjust for the correlation among measurements within the same woman. The main effects in the model will be time (0, 6, 12), treatment group (metformin versus placebo), and the interaction between time and treatment group. The time parameter tests if there is a change among placebo treated women, while the group-by-time interaction tests if the change in metformin treated women differs from that in the placebo group. We expect that a simpler covariance structure, such as compound symmetry, will be adequate for repeated measurements within the same woman (since the measurements are equally spaced). Alternatively, we will compare correlation structures for the longitudinal measurements using Akaike's information criterion. All endpoints will be first assessed for normality, and transformations (such as a logarithmic transformation) will be used as necessary to reduce skewness prior to statistical analysis.

Secondary analyses will assess whether differences in the changes in FWR-MRI between the metformin and placebo groups remain after adjustment for potential explanatory variables, such as changes in body weight, systemic hormones, and cytokines. These explanatory variables will be added as independent predictors in the linear mixed-effects models. These analyses will be restricted to explanatory variables that have demonstrated statistically significant metformin effects across time.

For the metabolomic aim, pre- to post-intervention changes in all detectable compounds of known identify (currently comprising 397 biochemicals using the standard platforms offered by Metabolon, Inc) will be determined and compared between treatment groups using a two-sample t-test. An estimate of the false discovery rate (q-value) will be calculated (115) to take into account the multiple comparisons that normally occur in metabolomic-based studies. A low q-value ($q \leq 0.10$) is an indication of high confidence in a result. While a higher q-value indicates diminished confidence, it does not necessarily rule out the significance of a result. Other lines of evidence may be taken into consideration when determining whether a result merits further scrutiny. Such evidence may include a) significance in another dimension of the study, b) inclusion in a common pathway with a highly significant compound, or c) residing in a similar functional biochemical family with other significant compounds.

Exploratory analyses will assess whether the observed metformin-induced changes in metabolite features are correlated with changes in FWR-MRI, body weight/composition, systemic hormones, and cytokines. The correlation will be estimated using a Pearson correlation coefficient. It is expected that any changes in these variables in the placebo group will reflect random variation only. Thus, these analyses will be restricted to the metformin group to overcome dilution of the potential correlations by individuals in the placebo group.

Exploratory analyses also will explore the correlation between baseline metabolite features and FWR-MRI, systemic hormones, and cytokines. These analyses will be restricted to

the metabolite features that are members of a common pathway to reduce the potential for an inflated alpha level.

9.2 Sample Size Justification

Our sample size ensures adequate power to test all primary specific aims. We assume an initial sample size of 75 women per arm and allow the possibility of up to 20% dropout for the 12-month follow-up measurement. This results in at least 60 evaluable women per group. To justify the sample size, we use the simplified comparison of change between 12 months versus baseline (ignoring the 6 month measurement). For the FWR-MRI-derived change in breast density, we will be able to detect a decrease of 0.516 standard deviation (SD) units in the metformin treated women with 80% statistical power, assuming a two-sided α of 0.05. To estimate what this change would be in relation to breast density, we used the relationship between breast density and log(Fra50) based on our previous data. Based on an analysis of our cross-sectional data, the estimated SD of log(Fra50) is 2.40. Thus, a decrease of 0.516 SD units corresponds to a change of -1.2384 (-0.516*2.40). Based on the regression line, this is equivalent to a 10% decrease in breast density as determined by standard mammography. For the additional primary outcomes (such as systemic hormones, cytokines, and weight), we will be able to detect a difference of 0.516 SD units with 80% statistical power, assuming a sample size of 60 evaluable women per group. Based on our previous experience, we anticipate that 65% of the proposed cohort will provide sufficient NAF for metabolomic analysis, which results in 39 women per group (allowing for up to 20% drop-out). This will allow us to detect a difference of 0.643 SD units with 80% statistical power.

9.3 Interim Analysis

No formal interim statistical analyses are planned for this Phase II trial. Accrual, data collection, and any adverse events will be monitored on a regular basis.

10. Data and Safety Monitoring Plan

10.1 Protocol Risk Level

This trial has been designated as a medium risk study. Medium risk studies are intended to include all trials involving therapeutic intervention(s), which are not designated as high risk per NCI and the IND is not held by the investigator.

10.2 Identification of the DSMB Obligated for Oversight Responsibilities

The University of Arizona Cancer Center Data and Safety Monitoring Board (DSMB) will provide ongoing oversight for this trial semi-annually.

10.3 Identification of the Entity Obligated for Routine Monitoring Duties

Routine monitoring will be provided by the Quality Assurance/Quality Control (QA/QC) Program to ensure that the investigation is conducted according to protocol design and regulatory requirements.

10.4 Monitoring Progress and Data Review Process

Routine monitoring of participant data will be conducted at least every 6 months.
The first routine monitoring visit will include at a minimum:

Informed consent – 100% of cases enrolled;
Participant eligibility - 50% of cases, up to two participants;
Data review - 50% of cases, up to two participants.

All subsequent monitoring visits will consist of randomly selected participant cases based on current enrollment and include continuing review of previously selected cases, as applicable. A monitoring visit report and follow-up letter will be completed within approximately

two weeks of the routine monitoring visit; a copy will be maintained in the study file. A query/finding form or an electronic record will also be completed by the monitor to request additional source documentation, clarification, information or corrections to the CRF and/or regulatory records. The study coordinator or other applicable staff responsible for the study will be given a copy of this form or will be notified of the electronic record for resolution of queries/findings. The query/finding form will be maintained with a copy of the visit report for follow-up at the next monitoring visit.

The Principal Investigator will ensure the accuracy, completeness, legibility and timeliness of the data reported in the Case Report Form (CRF), or other acceptable data formats. Source documentation supporting the study data should indicate the participant's participation in the trial and should document the dates and details of study procedures, adverse events, and participant status. CRFs should be completed via the institutional database or other acceptable data formats. Trials using paper CRFs will have data entered with a black ball-point pen or typed. Corrections to the forms should not obscure the original entry and should be made by striking the incorrect information with a single line. Each strike should be accompanied by the initials of the corrector and the correction date. All participant forms and study files will be stored in a secure area limited to authorized staff. ***Note****:* Routine monitoring of regulatory documents and test article will be conducted at least annually. A process is in place to implement study closure when significant risks or benefits are identified.

11. Description and Reporting of Adverse Events

11.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a study participant. An AE does not necessarily have a causal relationship with the treatment or study participant. An AE can therefore be any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with participation in a study, whether or not related to that participation. This includes all deaths that occur while a participant is on a study.

A list of adverse events that have occurred or might occur (Reported Adverse Events and Potential Risks) can be found in Section 6.2, Pharmaceutical Information as well as the package insert.

All adverse events that occur after the informed consent is signed must be recorded whether or not related to study agent. All adverse events will be entered into the OnCore database.

AE Data Elements include:

- AE reported date
- AE Verbatim Term
- Common Terminology Criteria for Adverse Events v4.0 (CTCAE) AE term
- Event onset date and event ended date
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a Serious Adverse Event (SAE)
- Whether or not the subject dropped due to the event
- Action taken with the study agent
- Outcome of the event
- Comments

All AEs will be assessed according CTCAE Version 4 (http://ctep.cancer.gov/protocolDevelopment/electronicapplications/docs/ctcaev4.pdf).

AEs that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4.0 as stated below.

Grade	Severity	Description
1	Mild	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL**.
4	Life threatening	Life-threatening consequences; urgent intervention indicated.
5	Fatal	Death related to AE.

Activities of Daily Living (ADL)
*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
**Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

The possibility that the adverse event is related to study agent will be classified as one of the following: not related, unlikely, possible, probable, definite.

All AEs, including lab abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices and documented as such.

11.2 Serious Adverse Events

ICH Guideline E2A and Fed. Reg. 75,Sept. 29, 2010 define serious adverse events as those events, occurring at any dose, which meet any of the following criteria:

- Results in death
- Is life threatening (*Note: the term life-threatening refers to an event in which the patient 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).*
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- Is a congenital abnormality/birth defect
- Important medical events that may not result in death, be life-threatening or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed.

Note: A SAE may also be an important medical event, in the view of the investigator that requires medical or surgical intervention to prevent one of the outcomes listed above.

All serious adverse events, regardless of attribution, and any deaths will be reported within 24 hours of notification of the event to the DSMB Coordinator. All SAEs which meet the criteria for a reportable event, as defined below, will be reported to the University of Arizona Human Subjects Protection Program (HSPP) within 10 working days of the event date or receipt of notification of the event.

Reportable events must meet all three of the following criteria:

- Unanticipated or unexpected in nature, frequency or severity, AND
- Related or possibly related to participation in the research (there is a reasonable possibility that it is related), AND
- The event suggests that the research places participants or others at a greater risk of harm than was previously known or recognized (not already referenced in existing study documents, such as the protocol, consent form or package insert).

All serious adverse events will be processed by the DSMB Coordinator monthly for initial trend analysis and fully reviewed by the DSMB, every six months. The DSMB coordinator will review the SAE reporting process to confirm reporting requirements are met.

11.3 Plan for assuring data accuracy and protocol compliance

Routine study activity and safety information will be reported to the DSMB every six months, or more frequently if requested. These reports will include:

- Study activity, cumulative and for the period under review;
- Safety (narrative description on non-serious and serious adverse events);
- Predetermined protocol early stopping rules for efficacy/futility;
- Monitoring and protocol compliance;
- Comments;
- Attachments (AE data reviewed by the PI to compile the report, SAE letters and reports, results of any review(s), applicable correspondence with the HSPP or other regulatory agencies).

Data, safety and study progress will be reported to HSPP at least annually and funding agency, as applicable. The PI will immediately notify; in writing, the funding agency, if applicable, any action resulting in a temporary or permanent suspension of the study.

11.4 Process to implement study closure when significant risks or benefits are identified

There will be no formal stopping criteria. However, if periodic review performed by the UACC DSMB identified patterns of significant risks, a discussion of more frequent monitoring or early closure will be conducted between DSMB and the investigators.

12. Regulatory Considerations

12.1 Regulatory Board Review

The study protocol will be activated after review and approval by the University of Arizona Cancer Center Scientific Review Committee and the University of Arizona HSPP. Approval of this protocol by the HSPP will also include approval of the protocol consenting document, or Informed Consent Form (ICF) and the accompanying HIPAA consenting instrument.

12.2 Compliance with Protocol and Protocol Revisions

Study personnel will conduct the study as described in this protocol. Any protocol revisions made in amendments to the protocol will be approved by the HSPP prior to implementation, except where necessary to avoid imminent hazard to a study participant. If an amendment alters the study design or increases the potential risk to the participant, the ICF will be revised and submitted for approval to the HSPP. The revised ICF will be used to obtain consent from participants currently enrolled in the study or after withdrawal from the study if they are affected by the Amendment.

12.3 Participant Informed Consent

All study participants will sign the most current ICF that has been approved by the HSPP prior to enrollment on the study. Investigators will have ensured that participants are clearly and fully informed about the purpose, potential risks and any other critical issues regarding this clinical trial.

12.4 Data Management and Safety Monitoring

Participant safety and data integrity will be monitored by the UACC Data and Safety Monitoring Board in conjunction with the Quality Assurance/Quality Control Program (QA/QC). All participants registered to this study will be entered into the UACC database (OnCore) for accrual and treatment status tracking. Verification of consent, date of consent, and other relevant registration data will be tracked in OnCore. All adverse events will be entered into the OnCore database. All participant study files will be stored in a secure area limited to authorized staff.

The Principal Investigator will ensure the accuracy, completeness and timeliness of the data reported. Source documentation supporting the reported data should indicate the participant's participation in the trial and should document the dates and details of study procedures, adverse events, and participant status.

13. Financing, Expenses, and/or Insurance

Study procedures performed during study visits will be covered by the study budget. Research tests, including serum and tissue biomarker evaluations, will not be billed to the subject. Subjects may incur minimal out-of-pocket expenses for transportation but will not be charged for study agent or any study-related activities. Subjects will receive monetary compensation which they may use at their discretion for out of pocket cost such as transportation. If injury occurs, medical care will be provided and charged to the subject's insurer.

References

1. Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. JAMA 2012;307: 491-7.
2. Li CI, Malone KE, Daling JR. Interactions between body mass index and hormone therapy and postmenopausal breast cancer risk (United States). Cancer Causes Control 2006;17: 695-703.
3. Hunter DJ, Willett WC. Diet, body size, and breast cancer. Epidemiol Rev 1993;15: 110-32.
4. Kaaks R, Van Noord PA, Den Tonkelaar I, Peeters PH, Riboli E, Grobbee DE. Breast-cancer incidence in relation to height, weight and body-fat distribution in the Dutch "DOM" cohort. Int J Cancer 1998;76: 647-51.
5. van den Brandt PA, Spiegelman D, Yaun SS, *et al.* Pooled analysis of prospective cohort studies on height, weight, and breast cancer risk. Am J Epidemiol 2000;152: 514-27.
6. Palomba S, Falbo A, Zullo F, Orio F, Jr. Evidence-based and potential benefits of metformin in the polycystic ovary syndrome: a comprehensive review. Endocr Rev 2009;30: 1-50.
7. De Leo V, La Marca A, Orvieto R, Morgante G. Effect of metformin on insulin-like growth factor (IGF) I and IGF-binding protein I in polycystic ovary syndrome. J Clin Endocrinol Metab 2000;85: 1598-600.

8. Pawelczyk L, Spaczynski RZ, Banaszewska B, Duleba AJ. Metformin therapy increases insulin-like growth factor binding protein-1 in hyperinsulinemic women with polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol 2004;113: 209-13.
9. Diamanti-Kandarakis E, Kouli C, Tsianateli T, Bergiele A. Therapeutic effects of metformin on insulin resistance and hyperandrogenism in polycystic ovary syndrome. Eur J Endocrinol 1998;138: 269-74.
10. Moghetti P, Castello R, Negri C*, et al.* Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivity in polycystic ovary syndrome: a randomized, double-blind, placebo-controlled 6-month trial, followed by open, long-term clinical evaluation. J Clin Endocrinol Metab 2000;85: 139-46.
11. Goodwin PJ, Pritchard KI, Ennis M, Clemons M, Graham M, Fantus IG. Insulin-lowering effects of metformin in women with early breast cancer. Clin Breast Cancer 2008;8: 501-5.
12. Sharma PK, Bhansali A, Sialy R, Malhotra S, Pandhi P. Effects of pioglitazone and metformin on plasma adiponectin in newly detected type 2 diabetes mellitus. Clin Endocrinol (Oxf) 2006;65: 722-8.
13. Adamia N, Virsaladze D, Charkviani N, Skhirtladze M, Khutsishvili M. Effect of metformin therapy on plasma adiponectin and leptin levels in obese and insulin resistant postmenopausal females with type 2 diabetes. Georgian Med News 2007: 52-5.
14. Desilets AR, Dhakal-Karki S, Dunican KC. Role of metformin for weight management in patients without type 2 diabetes. Ann Pharmacother 2008;42: 817-26.
15. Zakikhani M, Dowling RJ, Sonenberg N, Pollak MN. The effects of adiponectin and metformin on prostate and colon neoplasia involve activation of AMP-activated protein kinase. Cancer Prev Res (Phila) 2008;1: 369-75.
16. Phoenix KN, Vumbaca F, Claffey KP. Therapeutic metformin/AMPK activation promotes the angiogenic phenotype in the ERalpha negative MDA-MB-435 breast cancer model. Breast Cancer Res Treat 2009;113: 101-11.
17. Liu B, Fan Z, Edgerton SM*, et al.* Metformin induces unique biological and molecular responses in triple negative breast cancer cells. Cell Cycle 2009;8: 2031-40.
18. Zhu Z, Jiang W, Thompson MD, McGinley JN, Thompson HJ. Metformin as an energy restriction mimetic agent for breast cancer prevention. J Carcinog 2011;10: 17.
19. Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Metformin and reduced risk of cancer in diabetic patients. BMJ 2005;330: 1304-5.
20. Bowker SL, Majumdar SR, Veugelers P, Johnson JA. Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin. Diabetes Care 2006;29: 254-8.
21. Libby G, Donnelly LA, Donnan PT, Alessi DR, Morris AD, Evans JM. New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes. Diabetes Care 2009;32: 1620-5.
22. Currie CJ, Poole CD, Gale EA. The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. Diabetologia 2009;52: 1766-77.
23. Bodmer M, Meier C, Krahenbuhl S, Jick SS, Meier CR. Long-term metformin use is associated with decreased risk of breast cancer. Diabetes Care 2010;33: 1304-8.
24. Decensi A, Puntoni M, Goodwin P*, et al.* Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis. Cancer Prev Res (Phila) 2010;3: 1451-61.
25. Vona-Davis L, Rose DP. Type 2 diabetes and obesity metabolic interactions: common factors for breast cancer risk and novel approaches to prevention and therapy. Curr Diabetes Rev 2012;8: 116-30.
26. Hadad S, Iwamoto T, Jordan L*, et al.* Evidence for biological effects of metformin in operable breast cancer: a pre-operative, window-of-opportunity, randomized trial. Breast Cancer Res Treat 2011;128: 783-94.
27. Niraula S, Dowling RJ, Ennis M*, et al.* Metformin in early breast cancer: a prospective window of opportunity neoadjuvant study. Breast Cancer Res Treat 2012;135: 821-30.
28. Bonanni B, Puntoni M, Cazzaniga M*, et al.* Dual effect of metformin on breast cancer proliferation in a randomized presurgical trial. J Clin Oncol 2012;30: 2593-600.
29. Algire C, Amrein L, Bazile M, David S, Zakikhani M, Pollak M. Diet and tumor LKB1 expression interact to determine sensitivity to anti-neoplastic effects of metformin in vivo. Oncogene 2011;30: 1174-82.
30. Phoenix KN, Vumbaca F, Fox MM, Evans R, Claffey KP. Dietary energy availability affects primary and metastatic breast cancer and metformin efficacy. Breast Cancer Res Treat 2010;123: 333-44.
31. Cuzick J, Warwick J, Pinney E, Warren RM, Duffy SW. Tamoxifen and breast density in women at increased risk of breast cancer. J Natl Cancer Inst 2004;96: 621-8.
32. McCormack VA, dos Santos Silva I. Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2006;15: 1159-69.
33. Boyd NF, Guo H, Martin LJ*, et al.* Mammographic density and the risk and detection of breast cancer. N Engl J Med 2007;356: 227-36.
34. Warner E, Lockwood G, Tritchler D, Boyd NF. The risk of breast cancer associated with mammographic parenchymal patterns: a meta-analysis of the published literature to examine the effect of method of classification. Cancer Detect Prev 1992;16: 67-72.

35. Boyd NF, Rommens JM, Vogt K, *et al.* Mammographic breast density as an intermediate phenotype for breast cancer. Lancet Oncol 2005;6: 798-808.
36. Martin LJ, Boyd NF. Mammographic density. Potential mechanisms of breast cancer risk associated with mammographic density: hypotheses based on epidemiological evidence. Breast Cancer Res 2008;10: 201.
37. Greendale GA, Reboussin BA, Slone S, Wasilauskas C, Pike MC, Ursin G. Postmenopausal hormone therapy and change in mammographic density. J Natl Cancer Inst 2003;95: 30-7.
38. Stuedal A, Ma H, Bjorndal H, Ursin G. Postmenopausal hormone therapy with estradiol and norethisterone acetate and mammographic density: findings from a cross-sectional study among Norwegian women. Climacteric 2009;12: 248-58.
39. Atkinson C, Warren R, Bingham SA, Day NE. Mammographic patterns as a predictive biomarker of breast cancer risk: effect of tamoxifen. Cancer Epidemiol Biomarkers Prev 1999;8: 863-6.
40. Decensi A, Robertson C, Guerrieri-Gonzaga A, *et al.* Randomized double-blind 2 x 2 trial of low-dose tamoxifen and fenretinide for breast cancer prevention in high-risk premenopausal women. J Clin Oncol 2009;27: 3749-56.
41. Yang WT, Lewis MT, Hess K, *et al.* Decreased TGFbeta signaling and increased COX2 expression in high risk women with increased mammographic breast density. Breast Cancer Res Treat 2010;119: 305-14.
42. Li T, Sun L, Miller N, *et al.* The association of measured breast tissue characteristics with mammographic density and other risk factors for breast cancer. Cancer Epidemiol Biomarkers Prev 2005;14: 343-9.
43. dos Santos Silva I, Johnson N, De Stavola B, *et al.* The insulin-like growth factor system and mammographic features in premenopausal and postmenopausal women. Cancer Epidemiol Biomarkers Prev 2006;15: 449-55.
44. Diorio C, Pollak M, Byrne C, *et al.* Insulin-like growth factor-I, IGF-binding protein-3, and mammographic breast density. Cancer Epidemiol Biomarkers Prev 2005;14: 1065-73.
45. Knowler WC, Barrett-Connor E, Fowler SE, *et al.* Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346: 393-403.
46. DPP. The 10-year cost-effectiveness of lifestyle intervention or metformin for diabetes prevention. Diabetes Care 2012;35: 723-30.
47. Kopans DB. Basic physics and doubts about relationship between mammographically determined tissue density and breast cancer risk. Radiology 2008;246: 348-53.
48. Yaffe MJ. Mammographic density. Measurement of mammographic density. Breast Cancer Res 2008;10: 209.
49. Law J, Faulkner K. Cancers detected and induced, and associated risk and benefit, in a breast screening programme. Br J Radiol 2001;74: 1121-7.
50. Stephenson GD, Rose DP. Breast cancer and obesity: an update. Nutr Cancer 2003;45: 1-16.
51. Plymate SR, Matej LA, Jones RE, Friedl KE. Inhibition of sex hormone-binding globulin production in the human hepatoma (Hep G2) cell line by insulin and prolactin. J Clin Endocrinol Metab 1988;67: 460-4.
52. Plymate SR, Jones RE, Matej LA, Friedl KE. Regulation of sex hormone binding globulin (SHBG) production in Hep G2 cells by insulin. Steroids 1988;52: 339-40.
53. McTernan PG, Anwar A, Eggo MC, Barnett AH, Stewart PM, Kumar S. Gender differences in the regulation of P450 aromatase expression and activity in human adipose tissue. Int J Obes Relat Metab Disord 2000;24: 875-81.
54. Del Giudice ME, Fantus IG, Ezzat S, McKeown-Eyssen G, Page D, Goodwin PJ. Insulin and related factors in premenopausal breast cancer risk. Breast Cancer Res Treat 1998;47: 111-20.
55. Hirose K, Toyama T, Iwata H, Takezaki T, Hamajima N, Tajima K. Insulin, insulin-like growth factor-I and breast cancer risk in Japanese women. Asian Pac J Cancer Prev 2003;4: 239-46.
56. Gunter MJ, Hoover DR, Yu H, *et al.* Insulin, insulin-like growth factor-I, and risk of breast cancer in postmenopausal women. J Natl Cancer Inst 2009;101: 48-60.
57. Kaaks R, Berrino F, Key T, *et al.* Serum sex steroids in premenopausal women and breast cancer risk within the European Prospective Investigation into Cancer and Nutrition (EPIC). J Natl Cancer Inst 2005;97: 755-65.
58. Micheli A, Muti P, Secreto G, *et al.* Endogenous sex hormones and subsequent breast cancer in premenopausal women. Int J Cancer 2004;112: 312-8.
59. Thomas HV, Key TJ, Allen DS, *et al.* A prospective study of endogenous serum hormone concentrations and breast cancer risk in premenopausal women on the island of Guernsey. Br J Cancer 1997;75: 1075-9.
60. Kabuto M, Akiba S, Stevens RG, Neriishi K, Land CE. A prospective study of estradiol and breast cancer in Japanese women. Cancer Epidemiol Biomarkers Prev 2000;9: 575-9.

61. Rosenberg CR, Pasternack BS, Shore RE, Koenig KL, Toniolo PG. Premenopausal estradiol levels and the risk of breast cancer: a new method of controlling for day of the menstrual cycle. Am J Epidemiol 1994;140: 518-25.
62. Jones J, Clemmons D. Insulin-like growth factors and their binding proteins: biological actions. Endocrine Review 1995;16: 3-34.
63. Schernhammer E, Holly J, Pollak M, Hankinson S. Circulating levels of insulin-like growth factors, their binding proteins, and breast cancer risk. Cancer Epidemiology, Biomarkers & Prevention 2005;14: 699-704.
64. Allen N, Roddam A, Allen D*, et al.* A prospective study of serum insulin-like growth factor-1 (IGF-1), IGF-II, IGF-binding protein-3 and breast cancer risk. British Journal of Cancer 2005;92: 1283-7.
65. Hankinson S, Schernhammer E. Insulin-like growth factor and breast cancer risk: evidence from observational studies. Breast Disease 2003;17: 27-40.
66. Tessitore L, Vizio B, Jenkins O*, et al.* Leptin expression in colorectal and breast cancer patients. Int J Mol Med 2000;5: 421-6.
67. Tessitore L, Vizio B, Pesola D*, et al.* Adipocyte expression and circulating levels of leptin increase in both gynaecological and breast cancer patients. Int J Oncol 2004;24: 1529-35.
68. Han C, Zhang HT, Du L*, et al.* Serum levels of leptin, insulin, and lipids in relation to breast cancer in china. Endocrine 2005;26: 19-24.
69. Miyoshi Y, Funahashi T, Tanaka S*, et al.* High expression of leptin receptor mRNA in breast cancer tissue predicts poor prognosis for patients with high, but not low, serum leptin levels. Int J Cancer 2006;118: 1414-9.
70. Chen DC, Chung YF, Yeh YT*, et al.* Serum adiponectin and leptin levels in Taiwanese breast cancer patients. Cancer Lett 2006;237: 109-14.
71. Mantzoros CS, Bolhke K, Moschos S, Cramer DW. Leptin in relation to carcinoma in situ of the breast: a study of pre-menopausal cases and controls. Int J Cancer 1999;80: 523-6.
72. Coskun U, Gunel N, Toruner FB*, et al.* Serum leptin, prolactin and vascular endothelial growth factor (VEGF) levels in patients with breast cancer. Neoplasma 2003;50: 41-6.
73. Sauter ER, Garofalo C, Hewett J, Hewett JE, Morelli C, Surmacz E. Leptin expression in breast nipple aspirate fluid (NAF) and serum is influenced by body mass index (BMI) but not by the presence of breast cancer. Horm Metab Res 2004;36: 336-40.
74. Chu NF, Makowski L, Hotamisligil GS, Rimm EB. Stability of human plasma leptin concentrations within 36 hours following specimen collection. Clin Biochem 1999;32: 87-9.
75. Groschl M, Wagner R, Dorr HG, Blum W, Rascher W, Dotsch J. Variability of leptin values measured from different sample matrices. Horm Res 2000;54: 26-31.
76. Klein S, Coppack SW, Mohamed-Ali V, Landt M. Adipose tissue leptin production and plasma leptin kinetics in humans. Diabetes 1996;45: 984-7.
77. Thomas T, Burguera B, Melton LJ, 3rd*, et al.* Relationship of serum leptin levels with body composition and sex steroid and insulin levels in men and women. Metabolism 2000;49: 1278-84.
78. Hu X, Juneja SC, Maihle NJ, Cleary MP. Leptin--a growth factor in normal and malignant breast cells and for normal mammary gland development.[see comment]. J Natl Cancer Inst 2002;94: 1704-11.
79. Somasundar P, Yu AK, Vona-Davis L, McFadden DW. Differential effects of leptin on cancer in vitro. J Surg Res 2003;113: 50-5.
80. Saxena NK, Vertino PM, Anania FA, Sharma D. leptin-induced growth stimulation of breast cancer cells involves recruitment of histone acetyltransferases and mediator complex to CYCLIN D1 promoter via activation of Stat3. J Biol Chem 2007;282: 13316-25.
81. Yin N, Wang D, Zhang H*, et al.* Molecular mechanisms involved in the growth stimulation of breast cancer cells by leptin. Cancer Res 2004;64: 5870-5.
82. Dieudonne MN, Machinal-Quelin F, Serazin-Leroy V, Leneveu MC, Pecquery R, Giudicelli Y. Leptin mediates a proliferative response in human MCF7 breast cancer cells. Biochem Biophys Res Commun 2002;293: 622-8.
83. Castellucci M, De Matteis R, Meisser A*, et al.* Leptin modulates extracellular matrix molecules and metalloproteinases: possible implications for trophoblast invasion. Mol Hum Reprod 2000;6: 951-8.
84. Rose DP, Gilhooly EM, Nixon DW. Adverse effects of obesity on breast cancer prognosis, and the biological actions of leptin (review). Int J Oncol 2002;21: 1285-92.
85. Sierra-Honigmann MR, Nath AK, Murakami C*, et al.* Biological action of leptin as an angiogenic factor.[see comment]. Science 1998;281: 1683-6.
86. Fischer S, Hanefeld M, Haffner SM*, et al.* Insulin-resistant patients with type 2 diabetes mellitus have higher serum leptin levels independently of body fat mass. Acta Diabetologica 2002;39: 105-10.
87. Wauters M, Considine RV, Yudkin JS, Peiffer F, De Leeuw I, Van Gaal LF. Leptin levels in type 2 diabetes: associations with measures of insulin resistance and insulin secretion. Horm Metab Res 2003;35: 92-6.

88. Leyva F, Anker SD, Egerer K, Stevenson JC, Kox WJ, Coats AJ. Hyperleptinaemia in chronic heart failure. Relationships with insulin.[see comment]. Eur Heart J 1998;19: 1547-51.
89. Matsubara M, Maruoka S, Katayose S. Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. Eur J Endocrinol 2002;147: 173-80.
90. Yang WS, Lee WJ, Funahashi T*, et al.* Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin.[erratum appears in J Clin Endocrinol Metab 2002 Apr;87(4):1626]. J Clin Endocrinol Metab 2001;86: 3815-9.
91. Stefan N, Stumvoll M. Adiponectin--its role in metabolism and beyond. Horm Metab Res 2002;34: 469-74.
92. Kang JH, Lee YY, Yu BY*, et al.* Adiponectin induces growth arrest and apoptosis of MDA-MB-231 breast cancer cell. Arch Pharm Res 2005;28: 1263-9.
93. Arditi JD, Venihaki M, Karalis KP, Chrousos GP. Antiproliferative effect of adiponectin on MCF7 breast cancer cells: a potential hormonal link between obesity and cancer. Horm Metab Res 2007;39: 9-13.
94. Brakenhielm E, Veitonmaki N, Cao R*, et al.* Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated endothelial cell apoptosis. Proc Natl Acad Sci U S A 2004;101: 2476-81.
95. Harvie M, Howell A, Vierkant RA*, et al.* Association of gain and loss of weight before and after menopause with risk of postmenopausal breast cancer in the Iowa women's health study. Cancer Epidemiol Biomarkers Prev 2005;14: 656-61.
96. Muti P, Stanulla M, Micheli A*, et al.* Markers of insulin resistance and sex steroid hormone activity in relation to breast cancer risk: a prospective analysis of abdominal adiposity, sebum production, and hirsutism (Italy). Cancer Causes Control 2000;11: 721-30.
97. Harris HR, Willett WC, Terry KL, Michels KB. Body fat distribution and risk of premenopausal breast cancer in the Nurses' Health Study II. J Natl Cancer Inst 2012;103: 273-8.
98. Sonnenschein E, Toniolo P, Terry MB*, et al.* Body fat distribution and obesity in pre- and postmenopausal breast cancer. Int J Epidemiol 1999;28: 1026-31.
99. Huang Z, Willett WC, Colditz GA*, et al.* Waist circumference, waist:hip ratio, and risk of breast cancer in the Nurses' Health Study. Am J Epidemiol 1999;150: 1316-24.
100. Lindon JC, Holmes E, Nicholson JK. Metabonomics in pharmaceutical R&D. FEBS J 2007;274: 1140-51.
101. Huo T, Cai S, Lu X, Sha Y, Yu M, Li F. Metabonomic study of biochemical changes in the serum of type 2 diabetes mellitus patients after the treatment of metformin hydrochloride. J Pharm Biomed Anal 2009;49: 976-82.
102. Thompson PA, Hsu CH, Green S*, et al.* Sulindac and sulindac metabolites in nipple aspirate fluid and effect on drug targets in a phase I trial. Cancer Prev Res (Phila) 2010;3: 101-7.
103. Grundy SM, Cleeman JI, Daniels SR*, et al.* Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005;112: 2735-52.
104. Crofford OB. Metformin. N Engl J Med 1995;333: 588-9.
105. Stumvoll M, Nurjhan N, Perriello G, Dailey G, Gerich JE. Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. N Engl J Med 1995;333: 550-4.
106. Khan AY, Macaluso M, McHale RJ, Dahmen MM, Girrens K, Ali F. The adjunctive use of metformin to treat or prevent atypical antipsychotic-induced weight gain: a review. J Psychiatr Pract 2010;16: 289-96.
107. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Zoli M, Melchionda N. Metformin in non-alcoholic steatohepatitis. Lancet 2001;358: 893-4.
108. Orchard TJ, Temprosa M, Goldberg R*, et al.* The effect of metformin and intensive lifestyle intervention on the metabolic syndrome: the Diabetes Prevention Program randomized trial. Ann Intern Med 2005;142: 611-9.
109. Bolen S, Feldman L, Vassy J*, et al.* Systematic review: comparative effectiveness and safety of oral medications for type 2 diabetes mellitus. Ann Intern Med 2007;147: 386-99.
110. de Jager J, Kooy A, Lehert P*, et al.* Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial. BMJ 2010;340: c2181.
111. Bailey CJ, Turner RC. Metformin. N Engl J Med 1996;334: 574-9.
112. Salpeter SR, Greyber E, Pasternak GA, Salpeter EE. Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus. Cochrane Database Syst Rev 2010: CD002967.
113. Salpeter SR, Greyber E, Pasternak GA, Salpeter Posthumous EE. Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus. Cochrane Database Syst Rev 2010: CD002967.
114. Li Z, Gmitro AF, Bilgin A, Altbach MI. Fast decomposition of water and lipid using a GRASE technique with the IDEAL algorithm. Magn Reson Med 2007;57: 1047-57.
115. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A 2003;100: 9440-5.