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CLINICAL TRIAL

A randomized controlled trial of metformin in women with components of metabolic syndrome: intervention feasibility and effects on adiposity and breast density

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Abstract

Purpose Obesity is a known risk factor for post-menopausal breast cancer and may increase risk for triple negative breast cancer in premenopausal women. Intervention strategies are clearly needed to reduce obesity-associated breast cancer risk.

Methods We conducted a Phase II double-blind, randomized, placebo-controlled trial of metformin in overweight/obese premenopausal women with components of metabolic syndrome to assess the potential of metformin for primary breast cancer prevention. Eligible participants were randomized to receive metformin (850 mg BID, $n=76$) or placebo ($n=75$) for 12 months. Outcomes included breast density, assessed by fat/water MRI with change in percent breast density as the primary endpoint, anthropometric measures, and intervention feasibility.

Results Seventy-six percent in the metformin arm and 83% in the placebo arm ($p=0.182$) completed the 12-month intervention. Adherence to study agent was high with more than 80% of participants taking ≥ 80% assigned pills. The most common adverse events reported in the metformin arm were gastrointestinal in nature and subsided over time. Compared to placebo, metformin intervention led to a significant reduction in waist circumference ($p<0.001$) and waist-to-hip ratio ($p=0.019$). Compared to placebo, metformin did not change percent breast density and dense breast volume but led to a numerical but not significant decrease in non-dense breast volume ($p=0.070$).

Conclusion We conclude that metformin intervention resulted in favorable changes in anthropometric measures of adiposity and a borderline decrease in non-dense breast volume in women with metabolic dysregulation. More research is needed to understand the impact of metformin on breast cancer risk reduction.

Trial registration ClinicalTrials.gov NCT02028221. Registered January 7, 2014, https://clinicaltrials.gov/ct2/show/NCT02028221

Keywords Metformin · Breast density · Anthropometric measures · Metabolic syndrome · Clinical trial

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Abbreviations

T2DM	Type 2 diabetes
AMPK	AMP-activated protein kinase
ER	Estrogen receptor
CBC/w diff	Complete blood count with differential
CMP	Comprehensive metabolic panel
AFFQ	Arizona Food Frequency Questionnaire
AAFQ	Arizona Activity Frequency Questionnaire
AE	Adverse event
CTCAE	Common Terminology Criteria for Adverse Events

Introduction

Obesity is increasingly becoming a worldwide epidemic. A recent report indicates that two-thirds of the U.S. adults are overweight or obese [1]. High adiposity is associated with an increased risk for post-menopausal breast cancer [2–4]. Conversely, studies reported a null or inverse association of obesity and premenopausal hormone receptor-positive breast cancer [3–5]. Harris et al. [6] showed that measures of abdominal obesity were associated with an increased risk for premenopausal ER-negative breast cancer. Similarly, Pierobon and Frankenfeld [7] demonstrated in a systemic review and meta-analysis that there is a significant association between triple negative breast cancer and obesity among premenopausal women. Intervention strategies are clearly needed to address obesity-associated breast cancer risk.

Metformin, a biguanide, has been used for decades as the first-line treatment for type 2 diabetes (T2DM). This drug is presently the most widely prescribed antihyperglycemic drug due to its favorable benefit-risk profile. Metformin controls hyperglycemia in T2DM by down regulation of gluconeogenesis through the activation of the AMP-activated protein kinase (AMPK) signaling pathway. In cancer cells, metformin has been shown to modulate key oncogenic signaling pathways through AMPK dependent and independent effects (reviewed by [8]). Preclinical studies demonstrated inhibitory effects of metformin on the growth of ER-positive and ER-negative breast cancer cells [9–11] and on the tumor growth of ER-negative xenograft models and human epidermal growth factor receptor-2 positive transgenic mammary carcinoma models [12, 13]. Some, but not all, case control and cohort studies investigating the relationship between diabetes and cancer have found that treatment with metformin appears to reduce the risk for breast cancer [14–17]. Although a recent systematic review and meta-analysis of 12 observational studies found no significant association between metformin exposure and incidence of breast cancer in patients with T2DM [18]. Given the retrospective nature of these studies and the possibility that the comparison treatments (such as sulfonylureas or exogenous insulin) may increase risk and studies assessed risk in individuals with clinical disease with known cancer risk associations, placebo-controlled intervention trials are clearly needed to assess the breast cancer preventive activity of metformin, particularly among those with pre-clinical disease. It is important to note that accumulating evidence suggests that T2DM and obesity share biological mechanisms for their association with breast cancer (reviewed by [19]). Therefore, metformin would have high potential for breast cancer risk reduction in individuals without diabetes with high adiposity. In addition, recent clinical and animal studies suggest that metformin may only exert tumor suppressive effects in metabolic phenotypes of high adiposity and metabolic syndrome [20–22].

We conducted a randomized, placebo-controlled clinical trial of metformin in overweight/obese premenopausal women with components of metabolic syndrome to assess the potential of metformin for primary breast cancer prevention. This manuscript reports intervention feasibility and effects on recognized and putative markers of breast cancer risk including breast density and anthropometric measures.

Materials and methods

Study design

The study was a Phase II randomized, double-blind, placebo-controlled trial conducted at the University of Arizona (Tucson, AZ, USA). Overweight/obese premenopausal women with components of metabolic syndrome were randomly assigned to receive metformin or placebo for 12 months to determine feasibility and effects on recognized and putative markers of breast cancer risk. Outcomes included breast density, assessed by fat/water MRI with change in percent breast density as the primary endpoint, anthropometric measures, and intervention feasibility. The study was approved by the Institutional Review Board at The University of Arizona.

Study drug

Metformin 850 mg tablets were purchased by the University of Arizona Cancer Center (UACC) Research Pharmacy from commercial sources [initially from Major Pharmaceuticals (Livonia, Michigan, USA) and from Mylan Pharmaceuticals (Canonsburg, Pennsylvania, USA) after the product line was discontinued by Major Pharmaceuticals]. Look-alike placebo tablets were manufactured by Pharm Ops, Inc (Phillipsburg, New Jersey, USA) according to good manufacturing practices using inactive pharmaceutical excipients. The study agents were dispensed by and returned to the UACC Research Pharmacy to retain the blind.

Study population

We recruited premenopausal women, 21–54 years of age, with no change in menstrual patterns for 6 months preceding the time of registration and a body mass index of 25 kg/m^2 or greater. Participants were required to have a waist circumference of ≥ 88 cm or ≥ 80 cm for Asian Americans or individuals with polycystic ovarian syndrome and at least one other component of metabolic syndrome [23]. These components included elevated triglycerides (≥ 150 mg/dL or on drug treatment for elevated triglycerides), reduced high-density lipoprotein cholesterol (< 50 mg/dL or on drug treatment for reduced high-density lipoprotein cholesterol), elevated blood pressure (≥ 130 mm Hg systolic blood pressure or ≥ 85 mm Hg diastolic blood pressure or on drug treatment for hypertension), or elevated fasting glucose (≥ 100 mg/dL). Women who were on any drug for diabetes were excluded. Other eligibility criteria have been detailed previously [24]. Written informed consent was obtained from all participants.

Study procedure

During the initial study visit, consented study participants underwent anthropometric measurements including weight, height, waist and hip circumferences, assessment of vital signs including body temperature, blood pressure and pulse, and a urine pregnancy test. Information on medical history and medication usage history, menstrual patterns/cycles, and breast cancer risk was collected. A fasting blood sample was collected for complete blood count with differential (CBC/w diff), comprehensive metabolic panel (CMP), lipids, and follicle-stimulating hormone and/or estradiol for women with uncertain menopausal status.

Participants who met all selection criteria returned for a baseline visit and underwent anthropometric measurements; collection of vital signs, fasting blood, urine, and nipple aspirate fluid (NAF); and review of menstrual cycles and medication usage. Participants completed the Arizona Food Frequency Questionnaire (AFFQ) and the Arizona Activity Frequency Questionnaire (AAFQ). Participants also underwent a fat/water MRI assessment of breast density. Participants who were unable to fit into the MRI scanner due to their large body size continued the study and underwent all other study procedures. Participants were provided an option to undergo ultrasound guided breast core needle biopsy. NAF, collected noninvasively as described previously [25], has been studied as a potential breast tissue surrogate for the discovery of biomarkers for breast cancer diagnosis or risk assessment [25–27]. NAF, breast biopsy, and urine samples were archived for additional biomarker analyses; data will be reported in the future when available. Frequencies of successful collection of NAF and breast biopsy are summarized in Supplementary Table 1.

Following completion of baseline evaluation, participants were randomized to receive metformin or placebo for 12 months. Participants were required to take one metformin (850 mg) or placebo tablet once daily with food for the first 4 weeks and one metformin (850 mg) or placebo tablet twice a day with food for the remaining treatment period. Participants were asked to keep an adverse event (AE) diary and menstrual cycle calendar throughout the study. In addition, participants were provided with an intake calendar for recording medication usage.

Participants returned to the clinic at months 3, 6, 9, and 12. For each study visit, participants underwent evaluation of urine pregnancy test, vital signs, menstrual cycles, medication usage, and adverse events. In addition, participants underwent collection of fasting blood, urine, and NAF and fat/water MRI at the months 6 and 12 visits. Breast core needle biopsy was repeated at month 6 for those who consented to this option. Participants also completed the AFFQ and AAFQ at the month 12 visit. Clinical laboratory measurements of CBC/w diff, CMP, and lipids were repeated at the month 12 visit.

Safety of the intervention was assessed by reported adverse events throughout the study and post-intervention changes in CBC/diff, CMP, and lipids. AEs were assessed according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

Adherence was assessed by returned pill count as well as by measurement of metformin concentrations in plasma samples collected at the 6- and 12-month visits. The plasma metformin concentrations were measured as described previously [28].

Fat/water MRI assessment of breast density

All MRI examinations were performed on a 3 T MR system (Magneton Skyra, Siemens Healthcare, Erlangen, Germany). The fat water separation was performed on the scanner computer using a vendor provided package, which generates the fat-only images, water-only images, and fat fraction maps (reflecting the percentage amount of fat signal in each voxel) along with R2* maps [29]. Apparent fat fraction maps were calibrated to reflect true volume fraction using a linear signal model, described previously [30]. The fraction of fibroglandular tissue of the entire breast is then calculated by dividing the total volume of fibroglandular tissue by the total volume of the breast. In order to obtain a density value directly comparable to mammographic density, the fraction of fibroglandular tissue is then converted to MR-based percent breast density according to our previous publication [30]. Fat and fibroglandular tissue volume were also calculated, representing non-dense and dense breast volume, respectively.

Statistical analysis

The primary study endpoint is change in percent breast density. The secondary study endpoints include absolute dense breast volume, non-dense breast volume, anthropometric measures, safety, and tolerability. Baseline measures were compared between the metformin and placebo groups using two-sample independent *t* tests for continuous variables and Chi-Square test for categorical data. Linear regression was performed to calculate correlation between log-transformed breast density measures and anthropometric measures. For longitudinal analysis, log-transformed study endpoints were analyzed with a linear mixed effects model for all the measured values across time and to adjust for the correlation between measurements within the same individual. The main effects included in the model were time (0, 6- and 12-month), treatment group (metformin versus placebo), and the interaction between time and treatment group. The time effect tested whether there was a change within the group and the group-by-time interaction tested whether the change in the metformin group was different from the placebo group. All models were adjusted for baseline values. Plots of the predicted values from the linear mixed effects models were generated using the mean of the fitted values (including estimates of the fixed and random effects). *p* values less than 0.05 were deemed statistically significant. The analysis of the secondary endpoints was not adjusted for multiple comparisons because of the exploratory nature of these endpoints. The results of the secondary endpoints have been interpreted cautiously. The analysis was performed using STATA version 15.1 (StataCorp 2017).

Results

The study opened to accrual in March 2014 and closed to accrual in November 2017. Nine hundred and eight-nine women were contacted for the study. 235 women were consented and assessed for eligibility. The CONSORT flow diagram is shown in Fig. 1. 84 women were excluded, with 71 not meeting study eligibility and 13 declining continued study participation. 151 were eligible and randomized to

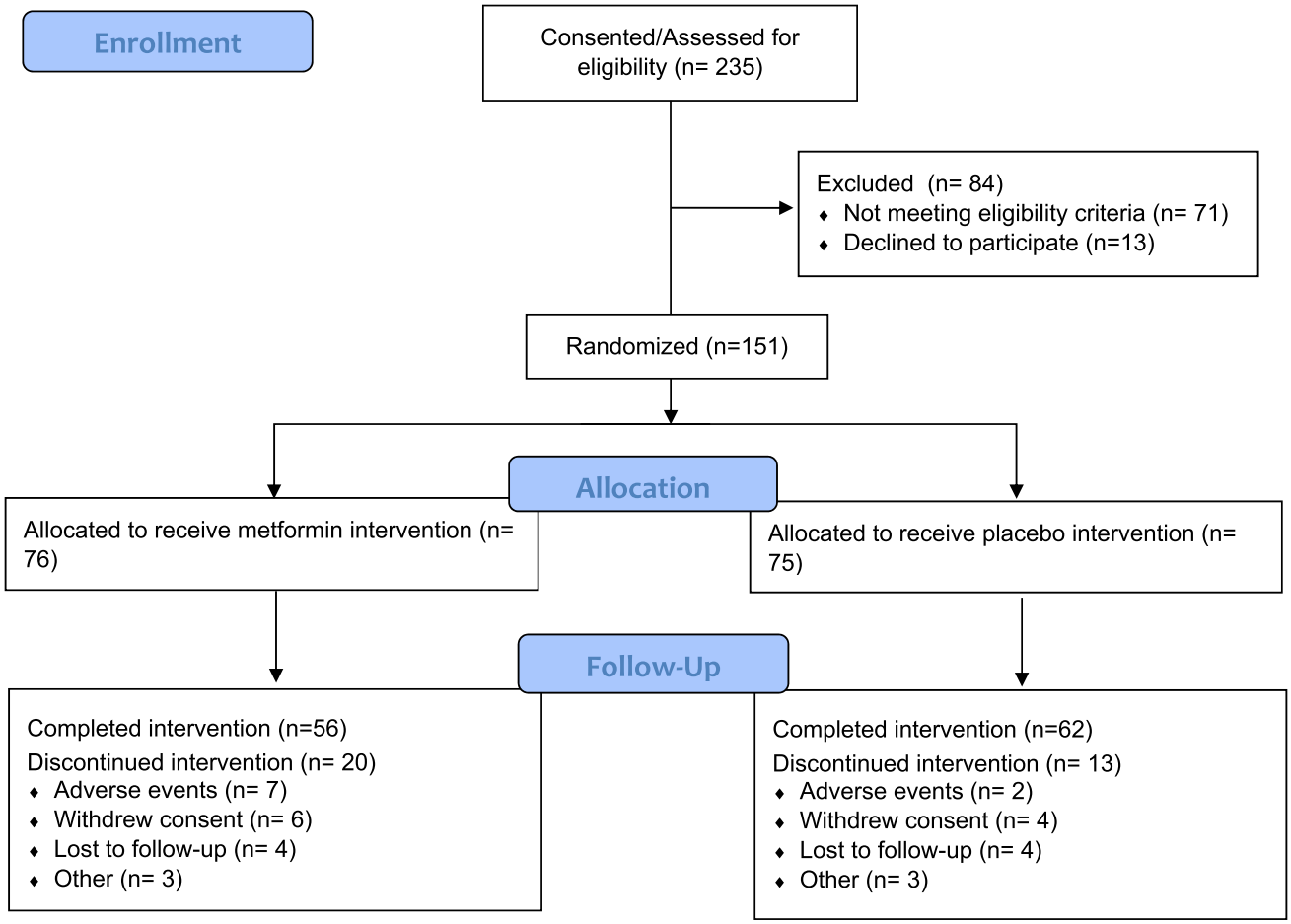

Fig. 1 Consort diagram

receive metformin ($n=76$) or placebo ($n=75$). Of the 151 randomized participants, 118 participants completed the 12-month intervention, 56 (74%) in the metformin arm, and 62 (83%) in the placebo arm ($p=0.182$). 20 participants in the metformin arm discontinued intervention due to adverse events ($n=7$), consent withdrawal ($n=6$), lost to follow-up ($n=4$), and other reasons ($n=3$). 13 participants in the placebo arm discontinued intervention due to adverse events ($n=2$), consent withdrawal ($n=4$), lost to follow-up ($n=4$), and other reasons ($n=3$).

The baseline characteristics of the randomized participants are summarized in Table 1. The average age among study participants was 39.5 ± 8.5 years. The average body mass index, body weight, waist circumference, hip circumference, and waist-to-hip ratio was 37.8 ± 6.5 kg/m^2, 101.9 ± 17.8 kg, 110.8 ± 12.4 cm, 125.7 ± 13.8 cm, and 0.88 ± 0.07, respectively. Because of the inclusion criteria, all participants had a large waist circumference as a component of metabolic syndrome. The most prevalent other components of metabolic syndrome were reduced HDL (65%), followed by elevated blood pressure (52%). Thirty percent

Table 1 Baseline characteristics of randomized study participants

	All ($n=151$)	Metformin ($n=76$)	Placebo ($n=75$)	p[d]
Demographics				
Age in years (mean ± SD)	39.5 ± 7.9	39.9 ± 7.9	39.2 ± 8.6	0.605
Race *n* (%)				0.729
White or Caucasian	126 (83)	64 (84)	62 (83)	
Black or African American	7 (5)	3 (4)	4 (5)	
Native Hawaiian or Other Pacific Islander	1 (1)	0 (0)	1 (1)	
Asian	1 (1)	0 (0)	1 (1)	
American Indian or Alaska Native	3 (2)	2 (3)	1 (1)	
More than 2	9 (6)	4 (5)	5 (7)	
Unknown	4 (3)	3 (4)	1 (1)	
Ethnicity *n* (%)				0.054
Hispanic or Latino	55 (36)	34 (45)	21 (28)	
Non-Hispanic or Latino	95 (63)	41 (54)	54 (72)	
Unknown	1 (1)	1 (1)	0 (0)	
Anthropometric measurements (mean ± SD)				
Body weight in kg	101.9 ± 17.8	101.4 ± 18.6	102.4 ± 16.9	0.730
Body mass index in kg/m^2	37.8 ± 6.5	37.3 ± 6.5	38.3 ± 7.2	0.403
Waist circumference in cm	110.8 ± 12.4	111.2 ± 13.0	110.4 ± 11.9	0.677
Hip circumference in cm	125.7 ± 13.8	125.6 ± 14.3	125.7 ± 13.5	0.957
Waist to hip ratio	0.88 ± 0.07	0.89 ± 0.07	0.88 ± 0.07	0.516
Components of metabolic syndrome *n* (%)				
Elevated triglycerides (≥ 150 mg/dL) or on drug treatment for it	50 (33)	22 (29)	28 (37)	0.274
Reduced HDL (< 50 mg/dL) or on drug treatment for it	98 (65)	50 (66)	48 (64)	0.818
Elevated BP (≥ 130 mm Hg systolic or ≥ 85 mm Hg diastolic) or on drug treatment for it	79 (52)	39 (51)	40 (53)	0.804
Elevated fasting glucose (≥ 100 mg/dL)	45 (30)	27 (36)	18 (24)	0.122
Components of metabolic syndrome				0.568
2	77 (51)	37 (49)	40 (53)	
≥ 3	74 (49)	39 (51)	35 (47)	
Breast density measures				
Dense volume (cm^3)	258.6 ± 136.2[a]	251.0 ± 148.0[b]	266.5 ± 123.8[c]	0.284
Non-dense volume (cm^3)	2648.2 ± 924.5[a]	2627.5 ± 935.4[b]	2669.6 ± 921.5[c]	0.779
Percent density (%)	23.6 ± 11.7[a]	23.2 ± 12.7[b]	24.1 ± 10.6[c]	0.396

[a] $n=110$

[b] $n=56$

[c] $n=54$

[d] Derived from two-sample independent *t* tests for continuous variables and Chi-Squared test for categorical variables

had elevated fasting glucose but were not on any drug for diabetes. The baseline characteristics were well balanced between the study arms. Of the 151 randomized women, we obtained fat/water MRI data from 110 participants at baseline. We were unable to obtain baseline MRI data from 37 participants due to larger body sizes (average BMI of 43.9 vs 35.8 kg/m^2 for those who could fit into the MRI scanner). The baseline MRI scans from four participants were unusable due to instrument issues. The overall baseline dense breast volume, non-dense breast volume, and percent breast density was 258.3 ± 136.2 cm^3, 2648.2 ± 924.5 cm^3, 23.6 ± 11.7%, respectively, and no statistical differences were observed in these baseline measures between groups.

Table 2 summarizes the adherence by pill count and by measurement of plasma metformin concentrations. In the metformin arm, 93% and 82% of the participants took ≥ 80% of the assigned pills for the baseline to 6-month and 6–12-month periods, respectively. In the placebo arm, 87% and 85% of the participants took ≥ 80% the assigned pills for the baseline to 6-month and 6–12 month periods, respectively. Adherence was also assessed by plasma metformin concentrations. In the metformin arm, 82% and 64% of the participants had detectable plasma metformin concentrations in samples collected at the 6- and 12-month visits, respectively. Surprisingly, 3% and 5% of the participants in the placebo arm had detectable plasma metformin concentrations in samples collected at the 6- and 12-month visits, respectively.

Safety of the intervention was assessed throughout the study. There were two Grade 3 diarrhea events possibly/probably related to the intervention, one reported in the metformin arm and the other in the placebo arm. All other related adverse events were Grade 1 and 2 in nature. The number and frequency of participants experiencing possibly/probably/definitely related Grades 1 and 2 adverse events over the 12-month intervention period are summarized in Supplementary Table 2. Most common adverse events observed in the metformin arm were gastrointestinal side effects. More participants in the metformin arm experienced diarrhea and nausea than those in the placebo arm (36.8% vs. 14.7% for diarrhea, $p = 0.003$, and 25.0% vs. 8.0% for nausea, $p = 0.008$). Interestingly, more participants in the placebo arm experienced flatulence than those in the metformin arm (12.0% vs. 2.6%, $p = 0.031$). The GI side effects reported in the metformin arm were more prevalent when the dose was first escalated from 850 mg QD to 850 mg BID and subsided for most participants over time (Supplementary Table 3).

Table 2 Compliance to study agent by pill count and by measurement of plasma metformin concentrations

	Metformin	Placebo
% of participants taken ≥ 80% of assigned pills		
Baseline to 6-month period	93	87
6–12 month period	82	85
% of participants with detectable plasma metformin concentrations		
6-month visit	82	3
12-month visit	64	5

Figure 2 illustrates the predicted means of anthropometric measures over time, derived from the linear mixed effects model, adjusted for baseline values. In the metformin arm, significant decreases were observed longitudinally in weight ($p = 0.003$), BMI ($p < 0.001$), waist circumference ($p < 0.001$), hip circumference ($p = 0.009$), and waist-to-hip ratio ($p = 0.034$). No significant changes were observed within the placebo arm**.** The changes in waist and waist-to-hip ratio following metformin intervention were significantly different than those observed in the placebo arm ($p < 0.001$ and $p = 0.019$, respectively). The changes in body weight and hip circumference following metformin intervention were numerically different from the placebo arm ($p = 0.061$ and $p = 0.057$, respectively). The longitudinal analysis was repeated restricting to individuals who have taken ≥ 80% pills, or to individuals who had detectable plasma metformin concentrations in the metformin arm and individuals who did not have detectable plasma metformin concentrations in the placebo arm. Restricted analysis by compliance determined by pill count did not affect the findings from the primary analysis. Restricted analysis by biomeasurement of compliance by plasma metformin concentrations showed significant effects from metformin on weight ($p = 0.034$), waist circumference ($p < 0.001$), hip circumference ($p = 0.047$), and waist-to-hip ratio ($p = 0.004$) compared to those in the placebo arm.

Figure 3 illustrates the predicted means of the breast density measures over time, derived from the linear mixed effects model, adjusted for baseline values. The non-dense breast volume was significantly decreased longitudinally ($p = 0.027$) in the metformin arm but the change was not significantly different from that observed in the placebo arm ($p = 0.077$). No significant change in dense breast volume and percent breast density was observed within each arm. There was no interaction between the treatment groups in dense breast volume and percent breast density. Restricted analysis by compliance determined by pill count and by plasma metformin concentrations attenuated the significance in decreased non-dense breast volume in the metformin arm ($p = 0.073$ and $p = 0.058$, respectively) but did not affect the findings from the other primary analyses.

Discussion

Obesity is a known risk factor for post-menopausal breast cancer [2–4] and may increase risk for triple negative breast cancer in premenopausal women [7]. Accumulating research

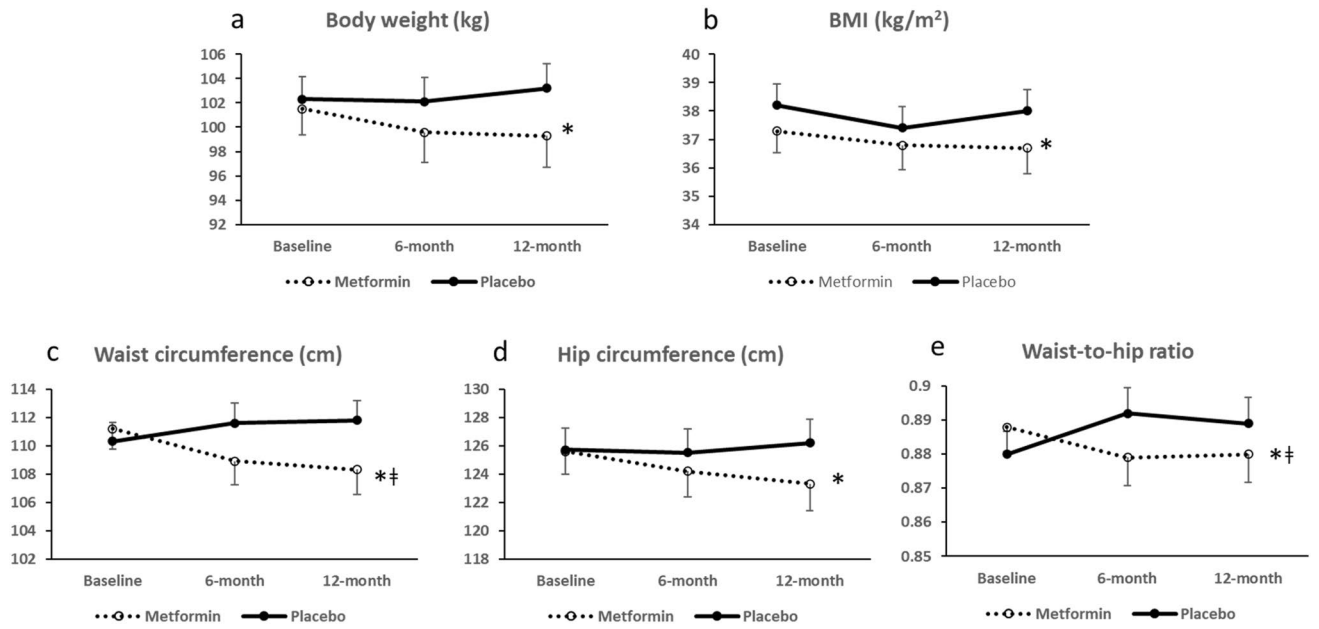

Fig. 2 Predicted means of anthropometric measures over time, derived from the linear mixed effects model, adjusted for baseline values. *Denotes statistical significance ($p < 0.05$) within group; ǂ denotes statistical significance ($p < 0.05$) between groups; **a** body weight; **b** BMI; **c** waist circumference; **d** hip circumference; **e** waist-to-hip ratio

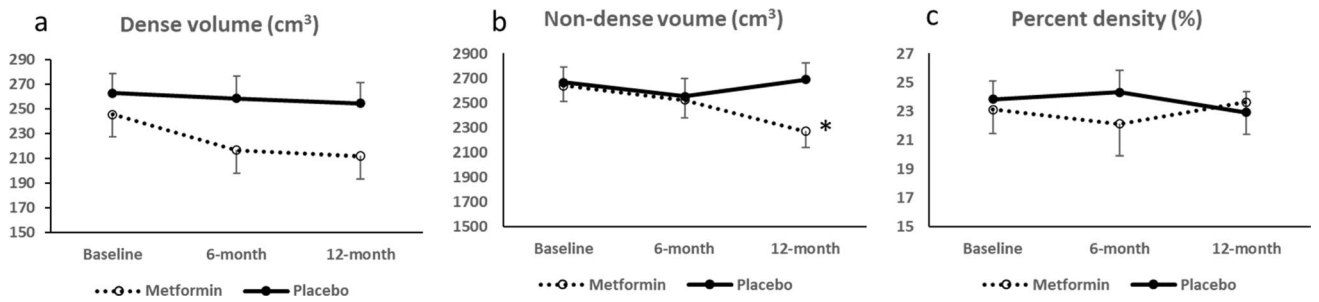

Fig. 3 Predicted means of the breast density measures over time, derived from the linear mixed effects model, adjusted for baseline values. *Denotes statistical significance ($p < 0.05$) within group; **a** dense volume; **b** non-dense volume; **c** percent density

supports the antitumor potential of metformin for reducing breast cancer risk in phenotypes of high adiposity and metabolic syndrome [20–22]. However, in order to achieve widespread use in primary breast cancer prevention in at-risk individuals, tolerance, adherence, and biological effects relative to the disease risk must be demonstrated. Here we reported a prospective randomized, double-blind, placebo-controlled trial in premenopausal women with components of metabolic syndrome to assess the potential of metformin for primary breast cancer prevention. We showed that more participants reported GI side effects in the metformin arm, mostly during the initial intervention period. Despite the initial GI side effects, the intervention completion rate was similar between the study arms. The metformin intervention had favorable effects on anthropometric measures of adiposity. Our study is the first reported randomized trial to assess the metformin effects on breast density and showed that metformin intervention did not change percent breast density and dense breast volume but led to a borderline decrease in non-dense breast volume in our study cohort.

In our trial, 74% in the metformin arm and 83% in the placebo arm completed the 12-month intervention. Participants had high agent adherence based on pill count, although the biomeasurement of adherence based on plasma metformin concentrations were lower than those assessed by pill count. Adherence assessment based on the returned pills reflects the adherence over the intervention period assessed. Adherence assessment based on plasma metformin concentrations reflects pill adherence proximal to the sample collection time. Based on the half-life of metformin (5 h) and the limit of metformin concentration measurement (2 ng/mL), detectable plasma metformin concentrations indicate pill adherence within 24 h prior to sample collection. We performed sensitivity analysis by restricting the analysis to those who

are adherence based on pill count (taken ≥ 80% pills) or those who had detectable metformin concentrations in the metformin arm. More significant changes in anthropometric measures were observed when restricting the analysis based on biomeasurement of adherence.

The major adverse events experienced were gastrointestinal disorders which included diarrhea and nausea, similar to those reported in the Diabetes Prevention Program (DPP), a large study that enrolled over 3,200 prediabetic participants with a BMI over 24 kg/m^2 and an intervention period of over 3.2 years [31]. The GI side effects attributed to metformin intervention may limit its clinical adoption for primary breast cancer prevention. As shown in Supplementary Table 3, the GI side effects in the metformin arm occurred more frequently when the dose was first escalated from 850 mg QD to 850 mg BID and subsided over time for most participants. The dose selected for this study is effective to prevent or delay type 2 diabetes for at least 15 years [32] and is used in ongoing clinical trials for secondary and tertiary breast cancer prevention. It is not known if this dose level is required for primary breast cancer prevention. Future studies may be warranted to determine if lower metformin doses or alternative metformin formulations would be effective in modulating breast cancer risk biomarkers and exhibit fewer side effects.

In our trial, metformin intervention reduced the anthropometric measures of adiposity in our cohort, with a significant decrease in waist circumference and waist-to-hip ratio and a numerical decrease in body weight and hip circumference. Similarly, weight loss was also observed at the end of year 1 during the DPP [33]. Prior clinical research suggested that metformin-induced weight loss is almost exclusively confined to reductions in adipose mass with little change in lean tissue [34, 35]. Metformin can affect energy expenditure through activation of AMPK which is a key regulator of mitochondrial biogenesis, fatty acid oxidation, and lipogenesis (reviewed by [36]). Metformin might also influence weight loss through reduced food intake owing to gastrointestinal related side effects. However, the change in energy intake assessed by baseline and post-intervention AFFQ was not different between arms (change of 10.9 ± 52.2% vs. − 7 ± 36.7% in the metformin vs. placebo arm, respectively, $p = 0.326$).

The study determined the metformin effect on the risk features of the breast by assessing the modulatory effect on breast density. Percent breast density, the relative extent of fibroglandular tissue in the breast, is the most studied mammographic density feature. It has consistently been shown to be strongly and positively associated with breast cancer risk [37–39]. Tamoxifen, the proven chemopreventive agent, has been shown to reduce percent mammographic density after 12–18 months of treatment [40, 41] and a 10% or greater reduction in percent mammographic breast density following 12–18 months of tamoxifen treatment has been associated with reduction in breast cancer risk [41]. Absolute breast density (dense breast area or volume), a measure of the absolute extent of fibroglandular tissue, is also positively associated the breast cancer risk [37–39]. Intriguingly, despite the positive association between adiposity and breast cancer risk, some studies [39, 42, 43] but not all [44, 45] have observed an inverse association between the amount of adipose tissue, reflected by non-dense breast area or volume, and breast cancer risk. We assessed breast density by fat/water MRI which provides 3-dimensional images of the breast without breast compression. This is especially relevant to obese women because obesity is positively correlated with increased compressed breast thickness on mammograms, which results in extensive overlapping tissue, lower radiographic image contrast, and inaccurate assessment of breast density [46]. We have previously used fat/water MRI derived percent breast density as an endpoint biomarker for evaluation of putative breast cancer preventive agents [47, 48]. In this study, we showed that the 12 months of metformin intervention did not change the percent breast density and dense breast volume but led to a numerical decrease in non-dense breast volume, reflecting a reduction in the extent of breast adipose tissue. Limited studies have examined the potential effects of metformin on breast density. A cohort study examining the association between diabetes and diabetes treatment and mammographic density showed that diabetic patients controlling the disease by diet or oral antidiabetic agents had a lower breast density, whereas taking insulin was associated with a higher breast density [49]. It is plausible that a longer intervention duration might be needed to observe metformin-induced changes in percent breast density. The implication of a decrease in the extent of breast adipose tissue on breast cancer risk in women with metabolic dysregulation is not known. Prior studies suggested that obesity is associated with breast adipose tissue inflammation [50–54] and extracellular matrix remodeling [55, 56] which can promote breast tumorigenesis. Future studies are planned to use the breast biopsies collected from this trial to examine the cellular and molecular risk biomarkers in the different breast tissue components to fully understand the metformin's effects on breast cancer risk.

Conclusion

We conclude that metformin intervention over 12 months resulted in favorable changes in anthropometric measures of adiposity and a borderline decrease in non-dense breast volume in women with metabolic dysregulation. More research is needed to understand the impact of metformin on breast cancer risk reduction.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10549-021-06355-9.

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Author contributions ET contributed to data acquisition, data analysis, data interpretation, and manuscript preparation, DVG contributed to data acquisition, data analysis, data interpretation, PC contributed to clinical evaluation, data acquisition, data analysis, data interpretation, SC contributed to data analysis, data interpretation, and manuscript revision, DR contributed to the study design, data analysis, data interpretation, and manuscript revision, JG-R contributed to data management and data analysis, CH contributed to fat/water MRI analysis, data interpretation, and manuscript revision, J-PG contributed to data acquisition, data interpretation, and manuscript revision, CT contributed to data interpretation and manuscript revision, MA contributed to the study design, data interpretation, and manuscript revision, JT contributed to data analysis and manuscript revision, LP contributed to data analysis and data interpretation, JM contributed to data interpretation and manuscript revision, MJ contributed to data interpretation and manuscript revision, H-HC contributed to the study design, clinical evaluation, data analysis, data interpretation, and manuscript preparation. All authors read and approved the final manuscript.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethical approval The study was approved by the Institutional Review Board at The University of Arizona.

Consent to participate Written informed consent was obtained from all participants.

Consent for publication Not applicable.

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