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Presurgical Trial of Metformin in Overweight and Obese Patients with Newly Diagnosed Breast Cancer

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Abstract

Introduction—We conducted a presurgical trial to assess the tissue-related effects of metformin in overweight/obese breast cancer (BC) patients.

Methods—Metformin 1,500 mg daily was administered to 35 nondiabetics with stage 0–III BC, body mass index (BMI) ≥ 25 kg/m². The primary endpoint was tumor proliferation change (i.e., ki-67). Tumor proliferation change was compared to untreated historical controls, matched by age, BMI, and stage.

Results—There was no reduction in ln(ki-67) after metformin ($p = .98$) or compared to controls ($p = .47$). There was a significant reduction in BMI, cholesterol, and leptin.

Conclusion—Despite no proliferation changes, we observed reductions in other relevant biomarkers.

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Ethical standards

All experiments in this manuscript comply with the current laws in the United States.

DECLARATION OF INTEREST

The authors declare that they have no conflict of interest. The authors alone are responsible for the content and writing of the article.

Keywords

Presurgical; Metformin; Ki-67; Hispanic; Breast cancer; Insulin resistance

INTRODUCTION

Metformin is a modulator of insulin resistance and benefits patients with diseases associated with insulin resistance, such as type II diabetes, polycystic ovarian syndrome, and HIV lipodystrophy (1–3). A significant amount of interest has been generated regarding the clinical role of metformin in breast cancer (BC) based on preclinical and epidemiologic investigations. Population-based studies report a lower rate of cancer in diabetic patients taking metformin compared with diabetic patients not taking metformin (4). In patients with BC, metformin users have a significantly better overall survival than nonmetformin users and nondiabetics (5). In a retrospective analysis, higher response rates to neoadjuvant systemic therapy have been reported in diabetic BC patients administered metformin as compared to those not receiving metformin or to nondiabetics (6). Laboratory-based studies demonstrate that metformin inhibits the growth of BC cell lines (7, 8), inhibits the inflammatory response and self-renewal of BC stem cells (9, 10), and enhances chemotherapy effectiveness in xenograft models (11). Proposed mechanisms of metformin's anti-cancer effects include reduction of serum insulin levels, direct modulation of cellular protein synthesis, and growth inhibition through effects in the AMPK/mTOR signaling pathway (12). These findings have led to several clinical trials evaluating metformin in women with BC.

Three presurgical metformin trials in BC have reported mixed results (13–15). In a presurgical or “window of opportunity trial,” patients with newly diagnosed cancer are exposed to an investigational drug for a limited time period between the time of diagnostic biopsy and surgical resection. Tumor proliferation, as measured by ki-67, is commonly evaluated in presurgical trials, as reduction in ki-67 after preoperative anti-estrogen therapy predicts BC outcome (16–20). Two metformin presurgical trials, one Canadian and one Scottish, observed a decrease in ki-67 (14, 15). However, in an Italian placebo-controlled, randomized trial, no change in tumor proliferation was identified after metformin use (13). In a preplanned subset analysis, reduction in tumor proliferation was restricted to patients with insulin resistance, as defined by an elevated homeostasis model assessment (HOMA) score.

We conducted a “window-of-opportunity” trial to evaluate metformin in overweight or obese patients with newly diagnosed BC. Overweight or obese patients were selected to enrich for an insulin resistant population. The primary outcome was to assess whether tumor proliferation was reduced in this population as compared to matched, untreated historical controls. Changes in fasting serum insulin and other blood markers in the insulin growth factor signaling pathway were also evaluated.

MATERIALS AND METHODS

Patients

We conducted an open-label, single-arm, presurgical trial with metformin at Columbia University Medical Center (CUMC) in New York, NY. Between October 2009 and August 2011, 35 nondiabetic women with a body mass index (BMI) ≥ 25 kg/m² and newly diagnosed histologically confirmed ductal carcinoma *in situ* (DCIS) ($n = 10$) or stage I–III primary BC ($n = 25$) were enrolled. Patients were required to have undergone a core needle biopsy, with the plan of a surgical excision at least 2 weeks after study enrollment. Women ≥ 21 years of age with no prior chemotherapy, radiation therapy, or surgery within 6 months of study entry were eligible. Exclusion criteria also included renal impairment with a creatinine > 1.4 mg/dL or a serious intercurrent medical illness. The study was approved by the CUMC institutional review board (clinicaltrials.gov identifier: NCT00930579). All patients signed informed consent in English or Spanish prior to study participation.

Treatment

Participants received metformin 1,500mg per day (500mg in the morning and 1,000mg in the evening) for at least 2 weeks between the initiation of study medication and surgery. Patients were evaluable if they had sufficient pre- and post treatment tumor tissue for analysis, and demonstrated at least 75% compliance with metformin, as measured by weekly telephone interviews and pill counts. During telephone interviews, safety and toxicity data were collected and scored, per the National Cancer Institute Common Terminology Criteria of Adverse Events, version 3.0. Adverse events were recorded in all patients who were administered metformin, regardless of completing the study. On day 0 (i.e., day in which metformin was dispensed to the patient) and the day prior to surgery, study participants underwent a physical exam and anthropometric measurements, including BMI and waist-to-hip circumference ratio. Metformin was to be taken until the night prior to surgery. After surgery, patients were treated with standard anti-cancer therapy.

In addition to the 35 patients enrolled, we collected tumor tissue on 35 untreated historical controls matched by age (± 10 years), stage (TNM), and BMI [± 5 kg/m² and within the same category: overweight (25.0–29.9 kg/m²) or obese (30.0 kg/m²-higher)]. The 35 controls were not being treated with metformin and were selected from the existing Herbert Irving Comprehensive Cancer Center breast database, which includes patients who previously consented to tumor tissue specimen research.

Clinical measurements and assessment of tumor proliferation

At baseline and the day prior to surgery, the following clinical measurements included height, weight, BMI, and waist/hip circumference for waist-to-hip ratio. Pathologic characteristics of tumors were collected, including tumor size, lymph node status, tumor grade, presence of lymphovascular invasion, and type of surgery.

On premetformin diagnostic core and postmetformin surgical resection formalin-fixed paraffin-embedded (FFPE) tumor tissue, estrogen receptor (ER), progesterone receptor (PR), and HER2 were assessed, per standard ASCO-CAP guidelines (21, 22). Sections at 4 μ m

thickness were cut for assessment of tumor proliferation, as measured by immunohistochemical staining of ki-67, per the International Ki-67 in BC Working Group recommendations for presurgical studies (23). Ki-67 was measured manually using the MIB-1 monoclonal antibody (1:200 dilution; DAKO, Carpinteria, CA). Controls were included in all batches. The ki-67 score was expressed as the percentage of positively staining cells among the total number of invasive cells in the evaluated area (23). When evaluable, 500 malignant invasive cells were scored. The pathologists (HH and AA) were blinded to whether the tumor tissue was from metformin-treated or historical controls. As a sensitivity analysis, correlation rates were conducted between ki-67 and tumor proliferation as measured by the reverse phase protein array (RPPA) proliferation cell nuclear antigen (PCNA) expression. RPPA was performed at the M.D. Anderson Cancer Center Functional Proteomics RPPA Core Facility (24, 25) and correlation between the proliferation indices were analyzed with the Pearson correlation coefficient.

Blood-based biomarkers

Fasting blood samples were collected at day 0 and the day prior to surgery. The following were evaluated: (a) insulin [Radioimmunoassay (RIA); Millipore Linco Research, Billerica, MA], (b) leptin (RIA), (c) adiponectin (RIA), (d) glucose [Cobas Integra 400 Plus Chemistry-analyzer; Roche Diagnostics, Indianapolis, IN], (e) lipids (Cobas), (f) IGF-1 [Immulate 1000; Siemens Healthcare Diagnostics, Deer-field, IL], and (g) IGFBP-3 (Immulate). All serum biomarkers were performed in the Biomarkers Core Laboratory of the Irving Institute for Clinical and Translational Research. Based upon the serum insulin and glucose level, the HOMA score was calculated: $\text{insulin } (\mu\text{U/mL}) \times \text{glucose (mmol/L)} / 405$ (26, 27). Blood biomarkers were not available for controls.

Statistical analysis

The primary endpoint was change in ki-67 staining between baseline and after metformin use in women with early invasive BC. Ki-67 results were transformed to the log-normal distribution [$\ln(\text{ki-67})$], per the international ki-67 recommendations (23). Based on the reduction of ki-67 with antiestrogen therapy in the presurgical setting (17), a sample size of 25 patients with invasive BCs provided 80% power to detect a 30% decrease in $\ln(\text{ki-67})$ from baseline to postmetformin values. Paired *t*-test was used to calculate changes in the $\ln(\text{ki-67})$ in tumors before and after treatment with metformin. Changes in $\ln(\text{ki-67})$ staining in the treatment cases and historical controls were compared using a two-sample *t*-test at level 0.05. Frequency distributions and summary descriptive statistics were used for all biomarkers. Paired *t*-tests were used to examine drug-related effects for each marker, including changes in anthropometric measures and blood-based biomarkers. Analysis of variance was performed to evaluate for differences in $\ln(\text{ki-67})$ based upon BMI and HOMA score. A probability of 5% was reported as significant, with trends to significance considered if 7%.

RESULTS

Patient population

From October 2009 to August 2011, 145 patients were screened; 70 were ineligible, and 40 declined to participate. The study flow is shown in Figure 1. Of the 35 patients enrolled, 33 completed the trial and were considered evaluable. Baseline patient and tumor characteristics are demonstrated in Table 1. The average age of evaluable patients was 53.3 years (SD: 11 years) and mean BMI was 31.2 kg/m² (SD: 5.3 kg/m²). Approximately half of patients treated with metformin were overweight, and half were obese. Approximately two-thirds of patients were postmenopausal. The majority of patients were Hispanic (85%). Among the Hispanic patients, 75% were Dominican and 20% were Puerto Rican.

Of the 33 evaluable patients, 9 had DCIS and 24 had invasive carcinoma (Table 1). As demonstrated in Figure 1, 3 patients with DCIS and 3 with invasive tumors did not have tumor at surgical resection or were identified to have different pathologic findings at resection as compared to the core biopsy. Of the invasive tumors, 80% of patients had in hormone receptor (HR) +/HER2-BC and 1 patient had triple negative (TN) BC. The majority of patients had moderately differentiated tumors (grade II: 67%) and no lymphovascular invasion (70%). The historical controls ($n = 34$) were well-matched, with an average age of 57.3 years and BMI of 31.2 kg/m². The immunophenotype of invasive tumor controls ($n = 24$) were comparable to matched metformin-treated patients, with 71% (17/23) HR+/HER2- and 3 TN BCs.

Metformin was administered for a median of 23 days (range: 8–64). The mean/median compliance was 100% as measured by pill count (range: 86%–138%, with 1 patient taking 1,500 mg po BID for the first 6 days). The most common side effects were grade I-II toxicities, including self-limited diarrhea, flatulence, abdominal pain, fatigue, and anorexia. Seven patients required dose reductions, and 1 patient discontinued metformin due to grade I vomiting. In Table 2, all Grade I toxicities with frequency > 5% and/or Grade II-III toxicities are reported. Grade III toxicities included abdominal pain ($n = 1$) and diarrhea ($n = 3$), with no grade IV/V events.

Changes in clinical characteristics and tumor proliferation

Of the evaluable metformin-treated patients, all but 1 patient had tumor tissue available for ki-67 immunohistochemical assessment. The baseline mean ki-67% was 21.5%, with a median of 11.5% (range: 3% to 35%; Table S1). For the invasive tumors ($n = 21$), the mean baseline absolute ki-67% was 23.0% (median: 10%), of which 10 patients had a ki-67 14%. There was a significant correlation between ki-67 and tumor proliferation as measured by RPPA expression of PCNA (correlation coefficient $r = 0.45$, $p = 7.96 \times 10^{-8}$). For statistical analysis, ki-67 was log-normalized. There was no significant difference in reduction of $\ln(\text{ki-67})$ between the premetformin core and postmetformin surgical tissue for all evaluable BCs (invasive and DCIS: $p = .98$) or invasive BC alone ($p = .43$; Table 3). When comparing changes in $\ln(\text{ki-67})$ between metformin-treated patients and matched controls, there was no significant difference in all BCs ($p = .47$). There was a reduction trend in the matched invasive BC controls compared to metformin-treated invasive BCs ($p = .06$). While no

significant difference was identified when comparing absolute differences in ki-67% in all BCs ($p = .11$), there was a significant reduction in invasive BC controls compared to metformin-treated tumors ($p = .05$) (Table S1). In addition, there was no significant difference between premetformin and postmetformin $\ln(\text{ki-67})$ in obese versus overweight women [$F(1,29) = 0.18, p = .67$] or based on HOMA score (<2.8 versus 2.8 : $F(1,29) = 1.54, p = .23$].

Given that 85% of the enrolled population was Hispanic, we conducted an unplanned subset analysis, exploring changes in tumor proliferation in Hispanic patients. For metformin treated Hispanic women ($n = 28$), there was no significant reduction in $\ln(\text{ki-67})$ after metformin use (mean change: -0.04 ; 95% CI: -0.55 to 0.47 ; $p = .87$). When compared to matched Hispanic historical control ($n = 15$), there was also no significant difference in $\ln(\text{ki-67})$ change (mean change: -0.21 ; 95% CI: -0.99 to 0.58 ; $p = .60$).

Changes in anthropometric measures and blood-based biomarkers

As seen in Table 4, there was a significant change in BMI after metformin ($p = .04$). Differences in waist/hip circumference were not significant. Fasting pre- and postmetformin serum was collected. Of the markers evaluated, there was a significant reduction in leptin ($p = .05$) and cholesterol ($p = .007$), specifically in LDL ($p = .004$). There was a trend toward a reduction in fasting insulin ($p = .06$) and HOMA score ($p = .07$). No significant changes were identified in fasting glucose, HDL, triglycerides, adiponectin, IGF-1, or IGFBP-3. In an unplanned, exploratory analysis of Hispanic metformin-treated patients, we observed a significant reduction in total cholesterol (mean change: -17.1 mg/dL, $p = .007$) and reduction in LDL (-20.5 mg/dL, $p = .004$). There was a trend toward reduction in HOMA score (-0.8 , $p = 0.07$) and insulin (-4.5 uIU/mL, $p = .07$). The other anthropometric and blood-based biomarkers were not significantly reduced after metformin in Hispanic women.

DISCUSSION

In this presurgical trial of overweight/obese patients, we did not identify a significant difference in breast tumor proliferation following metformin exposure. This lack of a significant reduction in tumor proliferation is consistent with the randomized, placebo-controlled, Italian metformin presurgical trial that also did not show an overall reduction in tumor proliferation after metformin use (13).

There are a number of variables beyond study design that may explain the different findings amongst the various presurgical studies (13–15). While the specific dosing of the metformin study varied across each trial, each limited the dose to 1,500 mg, except for the Scottish trial in which the maximum dose was 2,000 mg (14). While the majority of BCs in the other studies were primarily HR+/HER2–, the median ki-67 in the Italian trial was 19% (13). In the Canadian trial, the median ki-67 was 28% (15). In our sample set, the median ki-67 for the invasive BCs was 10%. Thus, our cohort of invasive BCs included less proliferative tumors. This proliferative rate was externally validated by the significant correlation between ki-67 and RPPA expression of PCNA. A number of factors may have led to the selection of slowly proliferative tumors, including the fact that most patients had small tumors and patients with larger tumors were likely selected for neoadjuvant chemotherapy.

The heterogeneity of breast tumor subtypes across these small presurgical studies may have led to differences in modulation of tumor proliferation.

Another important clinical characteristic that separates our trial from the other presurgical studies is that 85% of patients self-identified as Hispanic. In the other studies, primarily non-Hispanic White women were enrolled. This raises the question of whether there are host factors, such as ethnicity, that may affect the impact of metformin on BC biology. As compared to non-Hispanic white women, Hispanics have an increased risk of BC death, adjusting for important clinical factors, such as tumor stage, socioeconomic status, and treatment (28). In addition, Hispanics have higher rates of obesity and metabolic syndrome (29, 30).

Racial/ethnic differences have been reported in the response to metformin in managing diabetes (31, 32). Differences in insulin sensitivity and glucose effectiveness have been identified between various ethnicities (33). Also, it is possible that pharmacogenomic factors may be contributing. Ethnic differences in allele frequency have been reported in genes that can impact metformin transport, such as the *OCT1* and *HMATE1* genes (34). In an unplanned exploratory analysis, we did not observe a significant reduction in tumor proliferation in Hispanic women. Due to the small number of patients with other ethnicities, direct comparison with non-Hispanics is not possible.

In the four presurgical trials with metformin, reduction in ki-67 was selected as the primary outcome. While early presurgical modulation of ki-67 predicts BC outcome with anti-estrogen therapy in HR+ BCs, (16–19), the question remains as to whether ki-67 reduction should be chosen as the primary tissue-based marker in nonhormonal presurgical trials. In addition, the method of evaluating and analyzing ki-67 was dissimilar between these presurgical studies, including the choice of ki-67 antibody [NCL-L-Ki67-MM1 (Leica Microsystems) (14) versus MIB-1 (Dako) (13, 15), number of evaluated nuclei per invasive cell (ranging from approximately 600 to 40,000 nuclei), and whether to evaluate absolute differences in ki-67 (14, 15) or normalize these levels by conducting natural log transformation (13). In our study, we followed the ki-67 international recommendations (23), similar to the Italian randomized trial (13).

We identified a significant reduction in cholesterol and BMI, and a reduction trend in fasting insulin and HOMA score following at least a 2-week exposure to metformin. The decrease in cholesterol is consistent with other presurgical studies (12). At ASCO 2013, the preliminary 6 month changes in anthropometric measures and blood biomarkers were reported from MA.32, the randomized adjuvant trial comparing the invasive disease-free survival of patients with early-stage BC treated with metformin versus placebo in addition to standard systemic therapy (35). Consistent with our presurgical trial, significant differences in BMI and insulin were observed. In MA.32, reduction in glucose was also seen at 6 months (35). As opposed to the Italian trial, which demonstrated differences in tumor proliferation based on HOMA score, we did not observe a difference in those with HOMA score <2.8 or ≥2.8. This difference in findings may be due to our small sample size or possibly to host differences in our overweight/obese minority population. After metformin use, we observed a significant reduction in leptin ($p = .05$), which is important for body

weight regulation (36). Increases in leptin correlates with insulin resistance in women (37) and obesity (38). In MA.32, leptin was also significantly reduced after 6 months of metformin, consistent with our trial (35).

Strengths of our study include that our study is different than other trials in its anthropometric and ethnic makeup. In addition, we assessed a control arm, matching for important clinical variables to compare with changes in the metformin-treated patients. This study demonstrates the importance of evaluating a control arm, as a ki-67 reduction was seen, particularly among the invasive tumors. Weaknesses include limited tumor samples at the time of resection. The study was nonrandomized and has a small sample size. Statistical analysis directly comparing modulation of tumor proliferation between Hispanic and non-Hispanic white women was not possible given the limited cohort.

In conclusion, there was no significant reduction in tumor proliferation observed in this presurgical trial of metformin in obese or overweight patients. The goal of presurgical trials is to investigate predictive markers of biologic response and to generate hypotheses for subset analyses for future, larger clinical trials, such as MA.32. Given the differences identifying the effect of metformin between presurgical trials, host factors, including ethnicity and BMI, and tumor features, such as baseline ki-67, should be considered in future studies when evaluating the effects of metformin on tumor biology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Study flow diagram.

Table 1**Baseline Patient and Tumor Characteristics of Evaluable Metformin-Treated Patients**

Patient Characteristics	
Age (years), mean (SD)	56.3 (11)
Menopausal status, <i>n</i> (%)	
Premenopausal	10 (30%)
Perimenopausal	3 (9%)
Postmenopausal	20 (61%)
Ethnicity, <i>n</i> (%)	
Hispanic	28 (85%)
Non-Hispanic White	2 (6%)
Non-Hispanic Black	2 (6%)
Asian	1 (3%)
BMI (kg/m ²), <i>n</i> (%)	
BMI 25.0–29.9	19 (54%)
BMI 30.0–39.9	13 (37%)
BMI 40.0–higher	3 (9%)
Tumor characteristics	
Initial histology, <i>n</i> (%)	
DCIS	9 (27%)
Invasive carcinoma	24 (73%)
Invasive ductal carcinoma	20
Invasive lobular carcinoma	4
Initial size, mean (SD)	
DCIS	4 mm (4)
Invasive carcinoma	8 mm (4)
Immunophenotype, <i>n</i> (%)	
DCIS (<i>N</i> = 9)	
HR+	7 (78%)
HR–	2 (22%)
Invasive carcinoma (<i>N</i> = 24)	
HR+/HER2- ^a	19 (80%)
HR+/HER2+	2 (8%)
HR–/HER2+	2 (8%)
Triple negative	1 (4%)
Grade, <i>n</i> (%)	
DCIS	
I	0
II	2 (78%)
III	7 (22%)
Invasive	
I	2 (8%)

II	16 (67%)
III	6 (25%)
Lymphovascular invasion, <i>n</i> (%)	
Present	10 (30%)
Absent	23 (70%)
Type of surgery, <i>n</i> (%)	
Lumpectomy	27 (82%)
Mastectomy	6 (18%)

BMI = body mass index, HR = hormone receptor, HER2 = human epidermal growth factor receptor 2, DCIS = ductal carcinoma *in situ*, TN = triple negative.

^aTree discordant cases between core and surgical resection tissue: 1 TN core with HR+/HER2– resection tissue and 2 HR+/HER2– cores with TN resection tissue. Classification in table is based upon diagnostic core biopsies.

Table 2

Adverse Events

Adverse Event	All Grades, N (%)		
	1	2	3
Diarrhea	21 (60%)	5 (14%)	3 (9%)
Flatulence	15 (43%)	1 (3%)	
Nausea	11 (31%)		
Anorexia	10 (29%)		
Fatigue	10 (29%)		
Dizziness	8 (23%)		
Headache	8 (23%)		
Abdominal distention	7 (20%)		
Abdominal pain	7 (20%)	1 (3%)	1 (3%)
Taste alteration	6 (17%)		
Dry mouth	5 (14%)		
Constipation	4 (11%)		
Rash	4 (11%)		
Heartburn	3 (9%)		
Vomiting	3 (9%)		
Back pain	1 (3%)	2 (6%)	
Myalgia		2 (6%)	
Hemorrhoids		1 (3%)	

Table 3
Changes in Tumor Proliferation [In(ki-67)] in Evaluable Metformin-Treated Patients and Matched Historical Controls

	Tissue	Mean (SD)	Median (IQR)	Mean Before/ After Metformin (95% CI)	p-value*	Proportional : Treated vs. Control (95% CI)	p-value**
Metformin							
Invasive + DCIS (n = 32)	Core ^a	2.17 (1.60)	2.44 (1.10 to 3.55)	-0.006 (- 0.44 to 0.42)	0.98	-0.20 (- 0.75 to 0.35)	0.47
	Surgery ^b	2.165 (1.67)	2.51 (0.69 to 3.55)				
	Core ^a	2.12 (1.73)	2.30 (0.69 to 3.69)	0.15 (- 0.24 to 0.55)	0.43	-0.56 (- 1.14 to 0.02)	0.06
	Surgery ^b	2.27 (1.64)	2.30 (1.10 to 3.91)				
Control							
Invasive + DCIS (n = 34)	Core	2.31 (1.48)	2.30 (1.10 to 3.69)	-0.20 (- 0.56 to 0.15)	0.25		
	Surgery	2.04 (1.48)	2.30 (1.10 to 3.22)				
Invasive only (n = 24)	Core	2.85 (1.33)	3.22 (2.30 to 3.69)	-0.41 (- 0.85 to 0.04)	0.07		
	Surgery	2.36 (1.57)	2.71 (1.61 to 3.56)				

^a Premetformin tumor sample.

^b Postmetformin tumor sample.

* Paired *t*-test comparing changes within group (e.g., invasive premetformin-treated core versus postmetformin-treated surgical tissue).

** Two-sided *t*-test comparing changes between metformin-treated samples versus control.

Table 4Changes in Anthropometric and Blood Markers ($N = 32$) in Metformin Treated Patients

Variable	Before/After Metformin	Mean (SD)	Median (IQR)	Mean Before/After Metformin (95% CI)	p-value
BMI (kg/m^2)	Before ^a	30.88 (4.6)	29.35 (27.98 to 34.18)	-0.27 (- 0.52 to - 0.02)	* _{0.04}
	After ^b	30.61 (4.5)	29.35 (27.24 to 33.80)		
Waist/hip circumference	Before ^a	0.89 (0.06)	0.90 (0.85 to 0.92)	0.04 (- 0.003 to 0.067)	0.08
	After ^b	0.93 (0.06)	0.9 (0.89 to 0.95)		
Insulin (uIU/mL)	Before ^a	18.73 (14.83)	13.90 (9.08 to 24.00)	-4.12 (- 8.49 to 0.24)	** _{0.06}
	After ^b	14.61 (8.44)	12.70 (7.70 to 18.60)		
Glucose (mg/dL)	Before ^a	93.59 (11.54)	94.00 (86.75 to 101.20)	-0.06 (- 3.73 to 3.61)	0.97
	After ^b	93.53 (10.64)	89.50 (86.75 to 99.00)		
HOMA score	Before ^a	4.50 (3.9)	3.20 (1.90 to 5.55)	-1.11 (- 2.29 to 0.08)	** _{0.07}
	After ^b	3.39 (2.0)	2.90 (1.98 to 4.73)		
Cholesterol (mg/dL)	Before ^a	205.10 (49.07)	202.00 (166.80 to 221.50)	-16.50 (- 28.26 to - 4.74)	* _{0.007}
	After ^b	188.60 (43.17)	182.50 (157.00 to 224.80)		
HDL (mg/dL)	Before ^a	49.34 (11.61)	49.00 (41.00 to 58.00)	0.35 (- 3.70 to 4.40)	0.86
	After ^b	49.69 (13.98)	49.00 (40.50 to 53.00)		
LDL (mg/dL)	Before ^a	128.70 (38.41)	128.00 (99.75 to 149.50)	-18.50 (- 30.63 to - 6.43)	* _{0.004}
	After ^b	110.20 (38.00)	110.50 (82.25 to 141.20)		
Triglycerides (mg/dL)	Before ^a	126.00 (74.25)	104.00 (81.25 to 147.80)	14.60 (- 16.62 to 45.81)	0.35
	After ^b	140.60 (95.01)	118.0 (75.00 to 152.00)		
Leptin (ng/mL)	Before ^a	29.51 (15.16)	24.70 (16.27 to 43.08)	-1.65 (- 3.33 to 0.02)	* _{0.05}
	After ^b	27.86 (13.96)	24.50 (18.12 to 36.09)		
Adiponectin (ng/dL)	Before ^a	7362.00 (3199.55)	6850.00 (5375.00 to 8600.00)	-364.00 (- 780.49 to 52.37)	0.08
	After ^b	6998.00 (3160.30)	6300.00 (5525.00 to 8075.00)		
IGFBP-3 ($\mu\text{g}/\text{mL}$)	Before ^a	4.24 (0.64)	4.28 (3.97 to 4.60)	-0.05 (- 0.25 to 0.14)	0.58
	After ^b	4.19 (0.82)	4.31 (3.69 to 4.78)		

Variable	Before/After Metformin	Mean (SD)	Median (IQR)	Mean Before/After Metformin (95% CI)	p-value
IGF-1 (ng/mL)	Before ^a	135.82(56.85)	127.00 (99.00 to 156.25)	2.79 (- 7.20 to 13.87)	0.53
	After ^b	133.03 (40.06)	124.00 (101.25 to 161.75)		

BMI = body mass index, HOMA = homeostatic model assessment, HDL = high-density lipoprotein, LDL = low-density lipoprotein, IGFBP-3 = insulin-like growth factor binding protein 3, IGF-1 = insulin-like growth factor 1.

^a Premetformin measurement.

^b Postmetformin measurement.

* Paired *t*-test, significance defined as *p* .05.

** Paired *t*-test, trend toward significance defined as *p* .07.