

Effect of Different Doses of Metformin on Serum Testosterone and Insulin in Non-Diabetic Women With Breast Cancer: A Randomized Study

Carlo Campagnoli,¹ Patrizia Pasanisi,² Chiara Abbà,¹ Simona Ambroggio,¹ Nicoletta Biglia,³ Tiziana Brucato,¹ Raffaella Colombo,¹ Saverio Danese,⁴ Michela Donadio,⁵ Elisabetta Venturelli,² Gianna Zito,¹ Franco Berrino²

Abstract

This is a randomized controlled trial to test the effect of different doses of metformin in patients with breast cancer and without diabetes, with the aim of modifying the hormonal and metabolic parameters linked to breast cancer prognosis. Analysis of the results suggest that the dose of 1500 mg/d of metformin causes a significant reduction of insulin and testosterone serum levels.

Background: Serum levels of insulin and testosterone may affect both breast cancer (BC) incidence and prognosis. Metformin reduces hyperglycemia and insulin levels in patients with diabetes. In women without diabetes and with polycystic ovary syndrome, metformin lowers both insulin and testosterone levels. Patients with diabetes who are treated with metformin showed a lower risk of cancer; a protective effect of metformin also was observed for BC. Recently, studies on metformin use for prevention or treatment of BC have been proposed in patients who are not diabetic. The aim of the present study was to test the effect of different doses of metformin on serum levels of insulin and testosterone in those postmenopausal patients with breast cancer and without diabetes who have basal testosterone levels ≥ 0.28 ng/mL (median value). **Patients and Methods:** A total of 125 eligible women were initially invited to take metformin 500 mg/d for 3 months. The 108 women who completed the first 3 months were invited to continue the study with metformin 1000 mg/d (500 mg twice a day [b.i.d.]) for 1 month. The women were then randomized into 2 groups, and, for the subsequent 5 months, 1 group increased the dose by taking metformin 1500 mg/d (500 mg 3 times a day [t.i.d.]), and the other group continued with metformin 1000 mg/d (500 [b.i.d.]). **Results:** A total of 96 women completed the study: 43 women received 1500 mg/d, and 53 women received 1000 mg/d. The women who took 1500 mg/d showed a significant reduction of insulin level, HOMA-IR index (homeostasis model assessment-insulin resistance index), testosterone level, and free androgen index compared with women treated with 1000 mg/d. After treatment with 1500 mg/d, the insulin level decreased by 25% and the testosterone level decreased by 23%. **Conclusion:** Both these changes might have a prognostic importance.

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Keywords: Breast cancer, Insulin, Metformin, Randomized trial, Testosterone

¹Unit of Endocrinological Gynecology, Azienda Ospedaliera OIRM-Sant'Anna Torino

²Epidemiology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori di Milano

³Unit of Oncologic Gynecology, Azienda Ospedaliera Ospedale Mauriziano, Università degli Studi di Torino

⁴Oncology Unit, Azienda Ospedaliera Universitaria San Giovanni Battista di Torino

⁵Breast Unit, Azienda Ospedaliera Universitaria San Giovanni Battista di Torino, Italy

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Address for correspondence: Patrizia Pasanisi, MD, Department of Preventive and Predictive Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, 20133, Milan, Italy

E-mail contact: patrizia.pasanisi@istitutotumori.mi.it

Introduction

Markers of insulin resistance, such as metabolic syndrome^{1,2} and high serum levels of insulin^{3,4} and testosterone,⁵⁻¹⁰ may affect both breast cancer (BC) incidence and prognosis. Several drugs have been proposed in addition to diet and life-style interventions to reduce insulin levels and markers of insulin resistance.¹¹ Among these, metformin appeared the most convenient, because it is an inexpensive and fairly well-tolerated drug, with gastrointestinal discomforts as common adverse effects. A potentially dangerous toxicity is lactic acidosis, but the risk is essentially confined to patients affected by renal, hepatic, or cardiac insufficiency.¹² Metformin is a biguanide, which has been used to treat type 2 diabetes for more than 50 years, with doses between 1500 and 2000 mg/d. Metformin increases insulin sensitivity, inhibits liver neoglucogenesis by activating adenosine 5'-monophosphate-activated protein kinase (AMPK), and reduces hyperglycemia and the insulin level.¹³ Diabetes is associated with increased cancer incidence.¹³ However, observational studies and a recent meta-analysis showed that patients with diabetes who were treated with metformin had a significantly lower risk of developing cancer or had a lower cancer mortality.¹⁴⁻¹⁶ A protective effect of metformin also was observed for BC.^{14,17-19}

Metformin may reduce the cancer risk through 2 main mechanisms: by activating AMPK, thus mimicking the effect of calorie-energy restriction, which reduces all energy-consuming processes in the cells, including cell proliferation,²⁰ and by reducing insulin resistance and, therefore, insulin levels, which has a mitogenic effect on cancer cells.^{13,21} Metformin also is used in women with polycystic ovary syndrome (PCOS); it reduces testosterone levels, mainly through its lowering effect on insulin levels.^{22,23} A reduction of testosterone levels has recently been shown even in postmenopausal women with previous PCOS and/or hyperinsulinemia due to insulin resistance.²⁴ Several researchers have hypothesized to use metformin for prevention or treatment of BC also in persons without diabetes,^{19,20,25-27} and studies are actually ongoing.^{25,26,28,29} Up to now, however, there are no data on the effects of metformin in persons without diabetes and on the dose to be used as an anticancer agent. In principle, the "optimal" dose of metformin should be the minimum dose able to reduce insulin and other markers of insulin resistance and to minimize drug discomforts, which are dose-dependent and occur in 10%-25% of patients.^{22,23} Analysis of some data suggests that the dose of metformin 1000 mg/d is sufficient to significantly reduce both insulin and testosterone levels in women who are not obese and who have PCOS.^{30,31}

We now present the results of a randomized phase II study on postmenopausal patients with BC and without diabetes and with basal testosterone levels ≥ 0.28 ng/mL at a preliminary evaluation. The study was intended to test the effect of different doses of metformin on serum levels of testosterone, insulin, and other metabolic parameters linked to BC prognosis.

Patients and Methods

Patients

The women eligible for the study were (a) postmenopausal (nonsurgical) for at least 12 months, (b) aged < 70 years, (c) at least 6 months after surgery for BC, (d) not affected by type 1 or type 2 diabetes, (e) without previous diagnosis of cancer other than BC, (f)

not on chemotherapy or aromatase inhibitors for at least the previous 6 months (because of their possible effect on serum testosterone levels³²), (g) not on tamoxifen treatment for at least the previous 6 months or on tamoxifen treatment to be continued for at least 1 year, (h) not affected by conditions that contraindicate the use of metformin,¹² (i) not receiving treatment with statins, and (l) not receiving cimetidine, which may reduce the renal clearance of metformin.

Between 2008 and 2010, 541 potentially eligible patients who were referred to 4 hospitals in the Turin area (northern Italy) were examined for serum testosterone level as a potential prognostic indicator.^{5,10} (prebaseline selection measurement) (Figure 1). A total of 180 women with testosterone levels ≥ 0.28 ng/mL (the median value) were fully informed about the study aim and design. The patients received information about metformin treatment and its possible adverse effects, and were invited to participate in the study. A total of 125 women signed an informed consent and agreed to participate in the trial. The study was approved by the institutional review board and ethical committee of all collaborating institutes.

Study Design

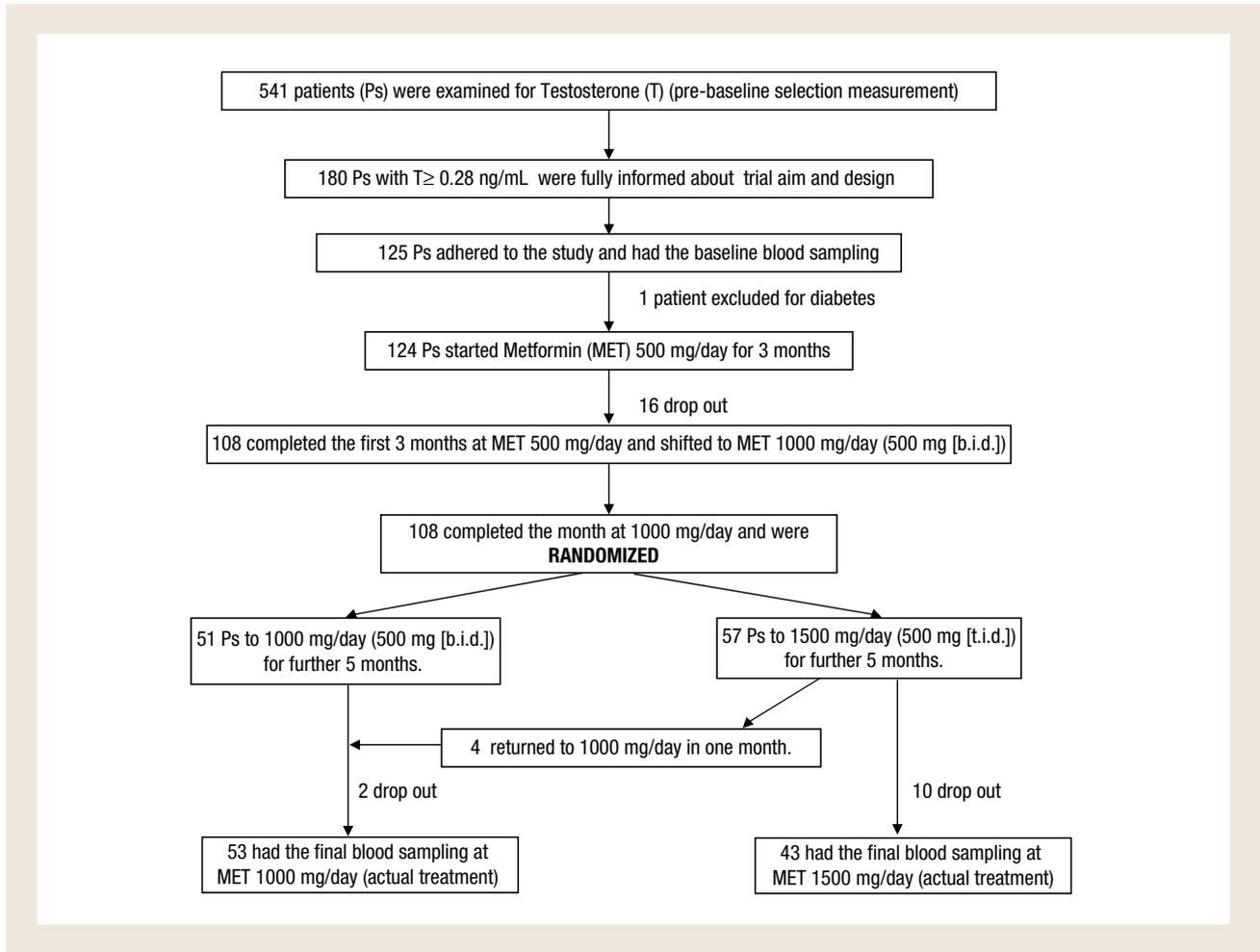
The aim of the study was to randomize women with serum testosterone levels higher than the median value of the distribution to receive either 1000 or 1500 mg/d of metformin to test the hormonal and metabolic effect of different doses. Women were initially invited to take metformin 500 mg/d for 3 months to minimize gastrointestinal discomforts that may occur with higher doses. The women who completed the first 3 months were invited to continue the study with metformin 1000 mg/d (500 [b.i.d.]) for 1 month. The women were then randomized into 2 groups, and, for the subsequent 5 months, 1 group was asked to increase the dose by taking metformin 1500 mg/d (500 [t.i.d.]), and the other group continued with metformin 1000 mg/d (500 [b.i.d.]). Women were randomized by using a stratified randomization design that included strata for age (< 58 years and ≥ 58 years) and baseline testosterone levels (< 0.44 and ≥ 0.44 ng/mL).

Fasting blood samples were collected before starting metformin treatment, after the first 3 months, and at the end of the study. Glycemia was measured with fresh plasma. Serum was stored at -80°C for the evaluation of insulin, testosterone, and sex hormone-binding globulin (SHBG) levels. At baseline, were collected full information on BC diagnosis, stage, treatment, and reproductive and menstrual histories. Height, weight, and waist circumference were measured at baseline and at follow-up visits at the third and ninth months. At each visit, the clinic physician collected updated information about health status and recorded drug adverse effects. Before starting metformin and at the third month, we checked serum creatinine and liver enzyme levels for safety evaluation.

Laboratory Measurements

To reduce intraindividual variation in hormone levels, blood samples were collected between 8 and 9 a.m., after overnight fasting, and were stored at -80°C . Previous studies showed that steroid and protein hormone levels are stable in serum preserved at low temperature.³³ Serum hormone levels were determined by using commercially available kits: radioimmunoassay kits from Orion Diagnostic (Turku, Finland) for testosterone; immunoradiometric assay kits from Farnos (Oulunsalo, Finland) for SHBG, and microparticle

Figure 1 Study Design



enzyme immunoassay kits from Abbot (Abbot Park, IL) for insulin. Baseline, third-, and ninth-month samples were analyzed in a single batch to reduce interassay variation. The coefficients of intra- and interassay variation in 8 replicates were 4.2% and 12.2% for a testosterone level of 0.37 ng/mL, 3.5% and 6.9% for an SHBG level of 48.5 nmol/L, and 2.5% and 5.1% for an insulin level of 8.1 μ IU/mL, respectively. The technicians who analyzed the serum samples were blinded to the randomization group of the patients.

Statistical Methods

The hormonal and metabolic variables of the women included in the study were approximately normally distributed. The homeostasis model assessment-insulin resistance (HOMA-IR) index was calculated by multiplying insulin level by glycemia concentration and dividing by 22.5.³⁴ Free androgen index (FAI) was estimated by dividing testosterone level (in nmol/L) by SHBG level (in nmol/L) and multiplying by 100. At baseline, the means of continuous variables in women treated with 1500 mg were compared with those of women treated with metformin 1000 mg/d by using the Student *t* test. The χ^2 test was used to compare frequencies and percentages in relationship to metformin treatment. Spearman correlation coefficients were computed to evaluate the cross-sectional relationship be-

tween hormonal and metabolic variables at baseline and longitudinal relationships among the changes in the different variables.

We analyzed the effect of the initial low dose of metformin (500 mg/d) on the parameters of interest by comparing their concentrations at baseline and at the third month. We used the Student *t* test to compare the effect of treatment in women randomized to receive 1000 and 1500 mg of metformin. Because 4 women randomized to the 1500-mg/d group after a few days shifted to the 1000-mg/d group because of alleged gastrointestinal adverse effects, we also carried out an analysis based on the actual treatment received. Because the differences were trivial, only the latter is reported.

The statistical analysis focused on changes in hormonal and other relevant variables, calculated as the difference between the end of the study (ninth month) and baseline values for each woman. The statistical significance of mean (SD) changes in the 1500-mg/d group compared with 1000-mg/d group was assessed by analysis of variance. We also performed separate analyses by baseline HOMA-IR index, ≤ 1.66 and > 1.66 (the median value of the distribution). All of the *P* values are 2 tailed. All analysis were performed by using the Strata 11 statistical package (Strata Corp, College Station, TX).

Results

Among the 125 patients with BC included in the trial, 1 patient was immediately excluded after the baseline blood examination due to previously undiagnosed diabetes. Sixteen women did not complete the first 3-month treatment with metformin 500 mg/d: 8 for gastrointestinal discomfort, 2 for a BC relapse, and 6 for nonclinical reasons. We observed a significant reduction of glycemia concentration ($2P < .01$) in the 108 patients who completed these first 3 months of treatment (data not shown). The testosterone levels also decreased significantly, but this was expected because of regression toward the mean. The 500-mg/d treatment did not substantially modify serum levels of insulin and SHBG. The 108 women were then invited to continue the study with metformin 1000 mg/d (500 [b.i.d.]) for 1 month. They were then randomized into 2 groups: 57 women were asked to increase the dose for the subsequent 5 months by taking metformin 1500 mg/d (500 [t.i.d.]), and 51 were asked to continue with metformin 1000 mg/d (500 [b.i.d.]).

Among the 57 women randomized in the 1500-mg/d arm, however, 4 decided to reduce their dose to 1000 mg/d after a few days of change in the treatment because of gastrointestinal discomfort. These 4 women eventually were added to the 1000-mg/d group for the present analysis. Four other women in the 1500-mg/d arm dropped out for gastrointestinal discomfort, 4 women dropped out in the first month after randomization for a BC relapse diagnosis, and 2 left for nonclinical reasons. Among the 51 women randomized in the 1000-mg/d arm, 1 dropped out for gastrointestinal discomfort and 1 dropped out because of BC relapse. As a result, 96 (88.9%) women completed the 9-month study, 43 women received 1500 mg/d, and 53 received 1000 mg/d (actual treatment).

Baseline clinical and anthropometric characteristics of the 96 women who completed the trial according to the actual metformin treatment are described in Table 1. The women who took 1500 mg/d were somewhat older (mean [SD], 58.4 ± 6.2 years vs. 56.1 ± 7.2 years) and leaner than those in the 1000-mg/d group, without, however, statistically significant difference. The 2 groups were comparable for the anthropometric parameters and tumor stage, and for the proportion of nodal metastasis at diagnosis and estrogen receptor (ER)-positive tumors. Six women among the 43 who took 1500 mg/d and 5 women among the 53 treated with 1000 mg/d had a history of minor irregularity in menstrual cycle before the age of 40 years. At baseline, body mass index (BMI) correlated with serum insulin levels ($r = .56$), glycemia concentrations ($r = .39$), and testosterone levels ($r = .20$). Testosterone levels significantly correlated with glycemia concentrations ($r = .21$). The correlations were similar in the 2 actual treatment groups.

The metabolic and hormonal parameters at baseline and the ninth month according to the actual metformin treatment (1000 vs. 1500 mg/d) are reported in Table 2. Baseline hormonal and metabolic parameters did not significantly differ between the 2 comparison groups. Women who took 1000 mg/d showed a significant reduction in body weight ($2P < .01$), BMI ($2P < .01$), waist circumference ($2P < .01$), glycemia concentration ($2P < .01$), and FAI ($2P = .03$) after 9 months of treatment. They showed substantially no change in serum insulin levels ($2P = .91$), a nonsignificant reduction in HOMA-IR index ($2P = .29$) and testosterone levels ($2P = .13$), and a nonsignificant increase of SHBG levels ($2P = .17$).

| Variable | Metformin 1000 mg/d (n = 53) | Metformin 1500 mg/d (n = 43) | P ^a |
|----------------------------------|------------------------------|------------------------------|----------------|
| Mean (SD) Age, Y | 56.1 ± 7.2 | 58.4 ± 6.2 | .09 |
| Mean (SD) Age At BC Diagnosis, Y | 50.7 ± 6.7 | 52.4 ± 6.7 | .24 |
| Mean (SD) Age At Menopause, Y | 48.2 ± 3.9 | 48.7 ± 3.7 | .48 |
| Tamoxifen, % Current Users | 49.1 | 39.0 | .33 |
| Tumor Stage, % < 2 cm | 71.7 | 62.8 | .35 |
| % Node+ | 30.2 | 27.9 | .81 |
| % ER+ | 78.4 | 82.5 | .63 |
| Mean (SD) Height, M | 1.61 ± 0.09 | 1.60 ± 0.07 | .55 |
| Mean (SD) Weight, kg | 68.2 ± 13.9 | 66.8 ± 15.5 | .63 |
| Mean (SD) BMI, kg/m ² | 26.1 ± 4.8 | 25.9 ± 5.2 | .82 |
| Mean (SD) Weight At Age 20 Y, kg | 50.0 ± 6.0 | 51.6 ± 6.7 | .24 |
| Mean (SD) Weight At Age 40 Y, kg | 58.7 ± 9.7 | 56.9 ± 9.9 | .38 |

Abbreviations: BC = breast cancer; BMI = body mass index; ER = estrogen receptor.
^a Differences were calculated by using the Student *t* test for continuous variables and the χ^2 test for frequencies and percentages comparison.

Women treated with 1500 mg/d showed a significant reduction in body weight ($2P < .01$), BMI ($2P < .01$), waist circumference ($2P = .02$), glycemia concentration ($2P < .01$), and FAI ($2P < .01$) after 9 months of treatment but also experienced a significant reduction in insulin levels ($2P < .01$), HOMA-IR index ($2P < .01$), and testosterone levels ($2P < .01$), and a significant increase of SHBG levels ($2P < .01$). When comparing the 2 groups, the women who took 1500 mg/d showed a greater and significant reduction of insulin levels, HOMA-IR index, testosterone levels, and FAI than the women treated with 1000 mg/d. After treatment with 1500 mg/d, insulin levels decreased by 25% between baseline and the ninth month, and testosterone levels decreased by 23%. Of the women treated with 1500 mg/d, 31 (72.1%) showed at least a 25% reduction of testosterone. The between-groups difference in testosterone levels change remained significant after controlling for age, baseline tertiles of BMI, and weight change ($2P = .01$). The differences in insulin and testosterone levels change remained significant after controlling for each other.

The effect of metformin 1500 mg/d in reducing testosterone and insulin levels was significant in both women who are lean and those overweight, whereas, metformin 1000 mg/d had no effect, either in women who are lean or in those overweight. Women treated with metformin 1500 mg/d showed a progressive reduction in serum insulin levels, HOMA-IR index, testosterone levels, and FAI, and an increase of SHBG levels with significant changes also between the third month and ninth month ($2P = .05$ for insulin level, $2P = .02$ for HOMA-IR index, $2P < .01$ for testosterone level, $2P < .01$ for FAI, and $2P = .01$ for SHBG levels) (data not shown).

Table 2 Metabolic and Hormonal Parameters at Baseline and at the Ninth Month by Actual Metformin Treatment Group

| Variable | Metformin, 1000 mg/d, mean (SD) (n = 53) | | Metformin, 1500 mg/d, mean (SD) (n = 43) | | P ^a |
|-------------------------------|--|-------------|--|-------------|----------------|
| | Baseline | 9th Mo | Baseline | 9th Mo | |
| Weight, kg | 68.2 ± 13.9 | 66.9 ± 13.5 | 66.8 ± 15.5 | 65.0 ± 16.1 | .28 |
| BMI, kg/m ² | 26.1 ± 4.8 | 25.7 ± 4.8 | 25.9 ± 5.2 | 25.3 ± 5.5 | .34 |
| Waist Circumference, cm | 88.9 ± 12.6 | 86.6 ± 12.6 | 87.2 ± 13.4 | 86.0 ± 13.3 | .36 |
| Glycemia Concentration, mg/dL | 88.9 ± 10.9 | 84.9 ± 9.0 | 91.1 ± 13.5 | 85.3 ± 10.3 | .39 |
| Insulin Level, μIU/mL | 8.4 ± 3.8 | 8.3 ± 3.2 | 9.5 ± 5.8 | 7.3 ± 4.7 | <.01 |
| HOMA-IR Index | 1.87 ± 0.99 | 1.75 ± 0.71 | 2.22 ± 1.50 | 1.57 ± 1.07 | <.01 |
| Testosterone Level, ng/mL | 0.36 ± 0.14 | 0.34 ± 0.14 | 0.41 ± 0.18 | 0.31 ± 0.13 | <.01 |
| FAI | 2.4 ± 1.4 | 2.2 ± 1.3 | 2.8 ± 1.9 | 2.0 ± 1.6 | <.01 |
| SHBG Level, nmol/L | 63.4 ± 27.3 | 67.1 ± 33.8 | 63.2 ± 32.1 | 71.9 ± 37.0 | .15 |

Abbreviations: BMI = body mass index; FAI = free androgen index; HOMA-IR = homeostasis model assessment-insulin resistance; SHBG = sex hormone-binding globulin.

^aAnalysis of variance between baseline and 9th mo, comparing metformin 1000 vs. 1500 mg/d.

The comparison between baseline and the ninth month for women treated with 1500 mg/d separately by HOMA-IR index at baseline (above and below the median value of population under study) are reported in Table 3. The women who took metformin 1500 mg/d showed a significant reduction of body weight, BMI, glycemia concentration, insulin levels, testosterone levels, and FAI, and a significant increase in SHBG levels, both in women with a HOMA-IR index less than or equal to the median value and in women with a HOMA-IR index greater than the median value, generally with a larger change in the group with great insulin resistance.

Compared with women treated with 1000 mg/d, the women who took 1500 mg/d showed a significantly greater reduction of insulin levels ($2P < .01$) and HOMA-IR index ($2P < .01$) in women with a HOMA-IR index less than or equal to the median value and a significantly greater reduction in testosterone levels ($2P < .01$) and FAI ($2P = .01$) in women with a HOMA-IR index above the median value (data not shown). In this latter group, there also was a borderline significant difference in insulin levels ($2P = .06$) and HOMA-IR index reduction ($2P = .06$) (data not shown). The decrease in insulin and testosterone levels was greater with higher baseline levels. Among women treated with metformin 1500 mg/d, however, the decrease was significant, both for those with baseline levels above the median of the population under study (insulin levels from 15.4 to 9.9 μIU/mL, $2P < .01$; testosterone levels from 0.56 to 0.40 ng/mL, $2P = .00$), and those with baseline levels under the median (insulin levels from 5.4 to 4.4 μIU/mL, $2P = .03$; testosterone levels from 0.27 to 0.24 ng/mL, $2P = .02$). Among women treated with 1000 metformin mg/d, insulin levels did not change, either among women with higher baseline levels or in those with lower levels, and testosterone levels decreased significantly only in women with higher baseline levels. When comparing the two actual treatment groups, the differences were significant in both women with high and with low baseline values (data not shown).

Discussion

We observed significant changes of metabolic and hormonal indicators of BC prognosis^{3,5,6,10,35} in postmenopausal patients with BC

and without diabetes who were treated with metformin. The main result was a significant decrease in insulin levels, HOMA-IR index, testosterone levels, and FAI in women treated with 1500 mg/d of metformin compared with women who took 1000 mg/d, who showed only minor changes in these variables. A low dose of metformin (500 mg/d), as used during the first 3 months, reduced glycemia concentration but did not reduce insulin levels. We also observed a significant reduction in testosterone levels, but this was expected because women included in the study were selected on the basis of their high testosterone levels (regression toward the mean). After randomization, any additional change in testosterone levels was observed only with the metformin dose of 1500 mg/d.

Analysis of results of several studies suggested that, in patients with diabetes, metformin acts as an anticancer agent, including for BC.^{20,26,36} This action may be mediated through the insulin-lowering effect of metformin.^{13,20,26,36,37} When insulin levels increase, insulin may bind and activate the related insulin-like growth factor receptor and the insulin receptor A, which have a potent mitogenic activity.^{20,21,26} High serum levels of insulin and insulin resistance increase the risk of cancer both in patients with diabetes^{13,20} and in those without diabetes.^{3,35} The improvement of insulin sensitivity and the reduction of insulin levels obtained by metformin open a new approach in management of BC.^{20,26} A single-arm phase II study of metformin treatment at 1500 mg/d of 22 patients with BC and without diabetes showed a 22.4% and 25.6% reduction of fasting insulin levels (mean [SD] baseline value, 9.9 ± 4.2 μIU/mL) and HOMA-IR index (mean [SD] baseline value, 2.2 ± 1.07), respectively.³⁶ Consistently, in our trial, the women treated with metformin 1500 mg/d experienced, on average, a 25% reduction of insulin levels (mean [SD] baseline value, 9.5 ± 5.8 μIU/mL) and a 29% reduction of the HOMA-IR index (mean [SD] baseline value, 2.2 ± 1.5) (Table 2). Furthermore, the treatment with metformin 1500 mg/d produced, on average, an 18% reduction of insulin levels and a 21% reduction of the HOMA-IR index also in women with baseline HOMA-IR index below the median value of 1.66 (Table 3).

Metformin and Serum Testosterone and Insulin Levels

Table 3 Comparison Between Baseline and 9th Month in Women Treated With Metformin 1500 mg/d by HOMA-IR Index

| Variable | Metformin, 1500 mg/d | | P ^a |
|---|----------------------|-------------------|----------------|
| | Baseline, mean (SD) | 9th Mo, mean (SD) | |
| Median HOMA-IR Index ≤ 1.66 (n = 21) | | | |
| Weight, kg | 60.9 ± 8.6 | 58.1 ± 7.8 | <.01 |
| BMI, kg/m ² | 23.5 ± 3.2 | 22.5 ± 2.9 | <.01 |
| Waist circumference, cm | 80.9 ± 9.1 | 79.3 ± 7.2 | .18 |
| Glycemia concentration, mg/dL | 83.9 ± 9.9 | 80.2 ± 6.8 | .05 |
| Insulin level, μIU/mL | 5.6 ± 1.9 | 4.6 ± 2.5 | .03 |
| HOMA-IR index | 1.13 ± 0.36 | 0.90 ± 0.46 | .02 |
| Testosterone level, ng/mL | 0.36 ± 0.17 | 0.29 ± 0.14 | .01 |
| FAI | 2.5 ± 1.8 | 1.7 ± 1.4 | <.01 |
| SHBG level, nmol/L | 64.3 ± 33.2 | 74.9 ± 37.4 | <.01 |
| Median HOMA-IR Index >1.66 (n = 22) | | | |
| Weight, kg | 73.4 ± 17.7 | 72.3 ± 18.6 | .02 |
| BMI, kg/m ² | 28.6 ± 5.2 | 28.2 ± 5.8 | .02 |
| Waist circumference, cm | 94.5 ± 13.2 | 93.2 ± 13.6 | .06 |
| Glycemia concentration, mg/dL | 97.6 ± 13.1 | 89.9 ± 10.8 | <.01 |
| Insulin level, μIU/mL | 13.6 ± 5.4 | 10.0 ± 4.8 | <.01 |
| HOMA-IR index | 3.3 ± 1.43 | 2.2 ± 1.09 | <.01 |
| Testosterone level, ng/mL | 0.46 ± 0.18 | 0.34 ± 0.12 | <.01 |
| FAI | 3.1 ± 2.0 | 2.3 ± 1.7 | <.01 |
| SHBG level, nmol/L | 63.2 ± 32.0 | 68.7 ± 37.2 | .05 |

Abbreviations: BMI = body mass index; FAI = free androgen index; HOMA-IR homeostasis model assessment-insulin resistance; SHBG = sex hormone-binding globulin.

^aComparison between baseline and 9th mo.

Results of a number of preclinical studies suggest that metformin may contrast BC also through direct effects, particularly by reducing protein synthesis and cell proliferation through the activation of AMPK.^{20,26} Usually, the metformin concentrations in these studies were 100-200 times higher than maximal plasma levels attainable in patients with diabetes, at a supraclinical dose, even when taking into account that the drug can concentrate in the tissues.³⁸ However, a most relevant direct effect of metformin, the one against cancer stem cells, was shown with concentrations below those attainable in tissues with the doses used in patients with diabetes.³⁹

A potential problem with the metformin treatment of cancer patients is that chronic exposure to a high dose of metformin, by acting as a caloric restriction mimetic, can stimulate cell intrinsic capacity for self-maintenance and repair.³⁸ The epidemiologic studies that showed lower cancer risk in patients with diabetes who are treated with metformin, however, are quite reassuring.¹⁴⁻¹⁸ However, metformin might be effective as an anticancer agent only in the presence of diabetes, hyperinsulinemia, or metabolic disorders.⁴⁰

Presently, there are several ongoing trials of patients with BC and without diabetes.^{25,38} A few studies are testing the effect of metformin before surgery on BC cell proliferation.^{28,38,41} A trial intends to evaluate the effect of metformin in human epidermal growth factor receptor 2-positive BC treated with neoadjuvant chemotherapy and trastuzumab.²⁹ The North American Breast Cancer Group is starting a phase III multicenter randomized trial to test metformin as an adjuvant BC therapy.²⁶ All of these studies use metformin doses in the range of 1500-2000 mg/d, as used in patients with diabetes.

The core question of our trial concerned the dose of metformin in patients with BC and without diabetes, with the aim of modifying the hormonal and metabolic parameters linked to the risk of BC recurrences,⁴² while minimizing drug adverse effects. Gastrointestinal discomforts due to metformin occur in 10%-25% of patients.^{22,23} Goodwin et al³⁶ reported a 12.5% drop out for gastrointestinal discomforts of women with BC who were treated with metformin 1500 mg/d. In our trial, 8 out 124 (6.4%) of women dropped out during the first 3 months of the study with metformin 500 mg/d for gastrointestinal discomforts. Among the women randomized in the metformin 1500 mg/d arm, 8 out 57 (14%) declared important gastrointestinal discomforts compared with 1 out 51 (2%) of women in the 1000-mg/d arm.

Results of a few studies suggested that metformin 1000 mg/d in women with PCOS without diabetes and who are not obese significantly reduces insulin and testosterone levels,^{30,31} without additional advantage if the dose is increased.³⁰ In our study, however, the metformin's lowering effect on insulin and testosterone levels was significant only with the dose of 1500 mg/d. We obtained the same results also when excluding 10 women with BMI > 30 kg/m² (data not shown).

Insulin stimulates the production of androgens and thus contributes to the pathogenesis of PCOS due to insulin resistance.^{22,23} Analysis of most data suggests that a high testosterone level is a marker of metabolic disorder also in postmenopausal women.^{24,42,43} Recently, a study showed that metformin 2000 mg/d in postmenopausal women with a history of PCOS and/or high levels of insulin and a high HOMA-IR index reduced the free testosterone level by 22%.²⁴ In our study, based on women without a history of PCOS but with baseline testosterone levels >0.28 ng/mL at a preliminary evaluation, only 24% of women showed insulin resistance (HOMA-IR index > 2.5),³⁴ but 89% showed levels of insulin >4 μIU/mL, which have been found to be associated with a higher risk of BC recurrences.³ A similar negative prognostic effect has been suggested for patients with BC and with a HOMA-IR index >1.2.³⁵ Among the women treated with metformin 1500 mg/d, those with HOMA-IR index higher than the median value of the distribution experienced the greatest reduction of testosterone level.

Epidemiologic studies showed that high serum levels of testosterone are associated with postmenopausal BC risk^{8,9} and BC recurrences.^{5,6,10} A recent prospective study suggested that high serum levels of free testosterone might differently affect BC according to the tumor ER status.⁷ The link between testosterone levels and tumor growth may be related either to the fact that testosterone is a marker of metabolic disorders or to the fact that it is converted into estradiol through the action of aromatases.^{7,9} Testosterone might also directly influence tumor growth through

the activation of the androgen receptor, which is expressed not only by almost all ER⁺ but also by a considerable proportion of ER⁻ tumors, but data are controversial.^{7,9,44,45}

The potential clinical impact of the 23% testosterone reduction in the women treated with metformin 1500 mg/d is strengthened by the significant increase in SHBG level (+14%) and the subsequent decrease of FAI (-30%). The use of metformin increased SHBG in women affected by PCOS⁴⁶ but had no effect in a previous study on menopausal women with insulin resistance.²⁴ In our study, the use of metformin 1500 mg/d increased SHBG level and decreased FAI both in women with a high and with a low HOMA-IR index (Table 3).

Conclusion

We know that metformin may theoretically reduce cancer risk, through 2 main mechanisms, by activating AMPK, thus mimicking the effect of calorie-energy restriction, which activates catabolic processes and reduces all energy-consuming processes in the cells, including cell proliferation, and by reducing insulin resistance and, therefore, insulin concentration in blood.²⁵ We designed our trial to test the effect of different doses of metformin in patients with BC who were not diabetic, with the aim of modifying the hormonal and metabolic parameters linked to the risk of BC recurrences⁴² while minimizing drug adverse effects. Our results showed that the dose of 1500 mg/d of metformin causes a significant reduction of insulin and testosterone serum levels. Because the metabolic and hormonal pattern affect BC risk and prognosis, both these changes might have a prognostic importance and open a novel approach to the management of BC.

Clinical Practice Points

- Diabetes is associated with increased cancer incidence, but several retrospective cohort studies showed that patients with diabetes who were treated with metformin had a significantly lower risk of developing cancer. A protective effect of metformin treatment also was observed for BC. Metformin is a biguanide used to treat type 2 diabetes for more than 50 years. It increases insulin sensitivity, inhibits liver neoglucogenesis, and reduces insulin and hyperglycemia. Metformin is also used to reduce circulating testosterone in women affected by PCOS, mainly through its lowering effect on insulin.
- We know that both insulin and testosterone levels may affect BC incidence and prognosis.
- Actually, after the encouraging observational data on patients with diabetes and preclinical results, there are several ongoing trials also in patients without diabetes, both in healthy women for prevention of BC and in patients with BC.
- We designed a randomized phase II study on postmenopausal patients with BC and without diabetes, with basal testosterone levels ≥ 0.28 ng/mL at a preliminary evaluation. The study was intended to test the effect of different doses of metformin on serum levels of testosterone and insulin, and other metabolic parameters linked to BC prognosis.
- The main result of our study was a significant decrease in insulin levels, HOMA-IR index, testosterone levels, and FAI in women treated with 1500 mg/d of metformin compared with women who took 1000 mg/d, who showed only minor changes in these vari-

ables. Because these parameters have been found associated with BC risk and prognosis, these results might have a clinical importance.

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Disclosure

The authors have stated that they have no conflicts of interest.

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