Metformin Decreases Circulating Androgen and Estrogen Levels in Nondiabetic Women With Breast Cancer

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Abstract

These are further data from a randomized controlled trial designed to test the effect of different doses of metformin in patients with breast cancer (BC) and without diabetes, with the aim of modifying the hormonal parameters linked to BC prognosis. A dose of 1500 mg/d of metformin causes significant reductions of the levels of free testosterone and estradiol.

Introduction: Diabetic patients treated with metformin have a lower risk of developing BC or a better BC prognosis. Metformin might reduce cancer growth through direct antiproliferative effects or through indirect mechanisms, particularly the reduction of insulin. In a randomized study on nondiabetic BC patients in natural menopause with high testosterone levels, we observed a significant decrease in insulin and in testosterone levels with metformin 1500 mg/d compared with 1000 mg/d. We present the results of a new analysis of our study on the effect of metformin on the bioavailability of sex hormones. **Patients and Methods:** One hundred twenty-four eligible women were initially invited to take metformin 500 mg/d for 3 months. The 108 women who completed the first 3 months continued the study using 1000 mg/d for 1 month. The women were then randomized into 2 groups, and, for the subsequent 5 months, 1 group increased the dose to 1500 mg/d, and the other group continued with 1000 mg/d. **Results:** Ninety-six women completed the study, 43 receiving metformin 1500 mg/day, and 53 receiving 1000 mg/day. The women receiving 1500 mg/d showed a greater and significant reduction of free testosterone (−29%) and estradiol (−38%), a borderline significant reduction of estrone and insulin-like growth factor-1, and a nonsignificant reduction of androstenedione. They also showed a nonsignificant increase of dehydroepiandrosterone sulfate. **Conclusion:** Metformin does not interfere with the production of dehydroepiandrosterone sulfate. Besides, it decreases estradiol levels, basically through the reduction of testosterone. These hormonal changes might have clinical relevance.

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Introduction

Observational studies have shown that diabetic patients treated with metformin (Metf) have a significantly lower risk of developing breast cancer (BC) than patients treated with other drugs.[1] A recent large prospective study reported a lower BC risk in diabetic women treated with Metf compared with nondiabetic women.[2] Diabetic women taking Metf also have a better BC prognosis,[3,4] especially if affected by HER2-positive BC.[5] Studies about the use of Metf in BC treatment also in nondiabetic women are ongoing.[6-8]

Metformin might reduce cancer risk through direct and indirect mechanisms. Direct actions are suggested by preclinical studies that show a decreased proliferation of all BC subtypes, mainly through the activation of adenosine-5'-monophosphate–activated protein

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kinase, which reduces all the energy-consuming processes in cells.[6] A major indirect mechanism is the reduction of insulin levels.[6,9] Insulin resistance and high serum levels of insulin are associated with an increased risk of BC and with BC relapses in diabetic and in nondiabetic women.[10-14] Actually, when insulin levels increase, insulin might bind and activate the related insulin-like growth factor (IGF) receptor and also insulin receptor A, which have potent mitogenic activities.[10,15] Further indirect mechanisms, possibly linked to insulin reduction, could be the modifications in sex hormone bioavailability. Actually, high serum levels of sex hormones, androgens and estrogens, are associated with an increased risk of BC and BC recurrences.[16-23] Therefore, the reduction of sex hormone bioavailability through the use of Metf might have a clinical effect.

We recently concluded a randomized phase II study on nondiabetic BC patients in natural menopause with high testosterone levels ($\geq$ 0.28 ng/mL) to test the effect of different doses of Metf on the serum levels of testosterone, insulin, and other metabolic parameters linked to BC prognosis.[24] In the women treated with 1500 mg/d Metf (compared with those receiving 1000 mg/d) we observed, together with significant decreases in insulin levels and in the homeostasis model assessment of insulin resistance (HOMA-IR) index, a significant reduction of testosterone levels and free androgen index.

After menopause, a relative excess of testosterone originates from the ovarian stroma.[25] However, ovaries contribute to the production of testosterone only for 25% to 45% of the total synthesis,[21,25-27] and androgens mainly derive from precursors produced in large quantities by the adrenal cortex, that is, dehydroepiandrosterone (DHEA) and particularly DHEA sulphate (DHEAS).[28] In the peripheral tissues, especially in the adipose tissue, these delta5 steroids are converted into delta4 androgens, that is, androstenedione and testosterone,[29,30] which, in turn, through aromatization in the adipose tissue, are the source of circulating estrogens, ie, estrone (E1) and estradiol (E2).[31,32] At present, there are no data about the effect of Metf on the levels of delta5 preandrogens and estrogens in BC patients.

The results of a further analysis of our randomized study[24] to test the effect of Metf on the serum levels of DHEAS, androstenedione, free testosterone, E1, and E2, are presented. We also evaluated the effect of Metf on IGF1, which has potent mitogenic activities[10] and influences the synthesis of androgens and the activity of estrogens in peripheral tissues.[31,33]

Patients and Methods

Patients

A detailed description of the study population has been published elsewhere.[24] Briefly, the women eligible for the study complied with these prerequisites: they (1) had been postmenopausal (nonsurgical) for at least 12 months; (2) were aged < 70 years; (3) had received surgery for BC at least 6 months before; (4) were not affected by type 1 or type 2 diabetes; (5) had not received a previous diagnosis of cancer other than BC; (6) had not been given chemotherapy or aromatase inhibitors for at least the previous 6 months; (7) had not been given tamoxifen treatment for at least the previous 6 months or were taking tamoxifen to be continued for at least 1 year; and (8) were not affected by conditions that contraindicate the use of Metf.

Among the 180 eligible women with serum testosterone levels $\geq$ 0.28 ng/mL (the median value), 124 signed an informed consent and were included in the trial. The study was approved by the Institutional Review Boards and the Ethical Committees of all collaborating institutes.

Study Design

The study was intended to test the effect of different doses of Metf in nondiabetic BC patients, with the aim of modifying the hormonal and metabolic parameters linked to the risk of BC recurrences[34] while minimizing drug side effects. The women were initially invited to take Metf 500 mg/d for 3 months, to minimize the gastrointestinal discomfort that might occur with higher doses. The 108 women who completed the first 3 months were invited to continue the study with Metf 1000 mg/d (500 mg twice per day) for 1 month. They were then randomized into 2 groups, and for the subsequent 5 months, the first group was asked to increase the dose, to Metf 1500 mg/d (500 mg 3 times per day), and the other group continued taking 1000 mg/d (500 mg twice per day).

Fasting blood samples were collected before starting Metf treatment, after the first 3 months, and at the end of the study. At baseline, we collected full information on BC diagnosis, stage, treatment, reproductive, and menstrual history. Height, weight, and waist circumference were measured at baseline, and at follow-up visits at the third and ninth month.

Laboratory Measurements

Blood samples were collected between 8 am and 9 am after overnight fasting, and stored at −80°C.

Serum samples were analyzed in batches by technicians blind to patient treatment. Each batch contained samples from 40 women plus 2 home-produced quality control samples; the 3 samples of every patient (baseline, third, and ninth month) were measured in the same batch.

Serum hormone levels were determined using commercially available kits: radioimmunoassay kits for testosterone, E2 (both Orion Diagnostic, Turku, Finland), E1 (DIAsource ImmunoAssays SA, Louvain-la-Neuve, Belgium), DHEAS, androstenedione, and IGF1 (Immunotech, Marseilles, France); immunoradiometric assay kits for sex hormone binding globulin (SHBG) (Farmos, Oulunsalo, Finland), and microparticle enzyme immunoassay kits for insulin (Abbot, Abbot Park, IL).

Based on the results obtained for the quality control samples, the interassay coefficients of variation were estimated to 12.2% for a mean of 0.37 ng/mL of testosterone, 6.9% for a mean of 48.5 nmol/L of SHBG 5.1% for a mean of 8.1 µIU/mL of insulin, 10.6% for a mean of 11.27 pg/mL of E2, 11.8% for a mean of 41.81 pg/mL of E1, 5.5% for a mean of 70.86 µg/dL of DHEAS, 10.8% for a mean of 0.96 ng/mL of androstenedione, and 6.2% for a mean of 121.88 ng/mL of IGF1.

Free testosterone was calculated from total testosterone, SHBG, and albumin (considering an average albumin concentration of 4.3 g/dL), using the method of Vermeulen et al[35] and a computer program (Free and Bioavailable Testosterone Calculator, developed at the Hormonology Department, University Hospital of Ghent, Belgium, and available at *http://www.issam.ch/freetesto.htm*).