

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

Carlo Campagnoli,<sup>1</sup> Franco Berrino,<sup>2</sup> Elisabetta Venturelli,<sup>2</sup> Chiara Abbà,<sup>1</sup> Nicoletta Biglia,<sup>3</sup> Tiziana Brucato,<sup>1</sup> Patrizia Cogliati,<sup>2</sup> Saverio Danese,<sup>4</sup> Michela Donadio,<sup>5</sup> Gianna Zito,<sup>1</sup> Patrizia Pasanisi<sup>2</sup>

## 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.

*Clinical Breast Cancer*, Vol. 13, No. 6, 433-8 © 2013 Elsevier Inc. All rights reserved.

**Keywords:** Dehydroepiandrosterone sulfate, Estradiol, IGF1, Randomized trial, Testosterone

<sup>1</sup>Unit of Endocrinological Gynecology, Ospedale Sant'Anna di Torino, Turin, Italy

<sup>2</sup>Epidemiology Unit, Fondazione IRCCS, Istituto Nazionale dei Tumori di Milano, Milan, Italy

<sup>3</sup>Unit of Oncologic Gynecology, Ospedale Mauriziano, Università degli Studi di Torino, Turin, Italy

<sup>4</sup>Oncology Unit, Ospedale Sant'Anna di Torino, Turin, Italy

<sup>5</sup>Breast Unit, Ospedale San Giovanni Battista di Torino, Turin, Italy

Submitted: Feb 18, 2013; Revised: Jul 8, 2013; Accepted: Aug 26, 2013

Address for correspondence: Patrizia Pasanisi, MD, MSc, Department of Preventive and Predictive Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Via Venezian, 1, 20133 Milano, Italy  
Fax: +390223903516; e-mail contact: [patrizia.pasanisi@istitutotumori.mi.it](mailto:patrizia.pasanisi@istitutotumori.mi.it)

## 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.<sup>1</sup> A recent large prospective study reported a lower BC risk in diabetic women treated with Metf compared with nondiabetic women.<sup>2</sup> Diabetic women taking Metf also have a better BC prognosis,<sup>3,4</sup> especially if affected by HER2-positive BC.<sup>5</sup> Studies about the use of Metf in BC treatment also in nondiabetic women are ongoing.<sup>6-8</sup>

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

## Metformin and Sex Hormones

kinase, which reduces all the energy-consuming processes in cells.<sup>6</sup> A major indirect mechanism is the reduction of insulin levels.<sup>6,9</sup> 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.<sup>10-14</sup> 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.<sup>10,15</sup> 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.<sup>16-23</sup> 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.<sup>24</sup> 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.<sup>25</sup> However, ovaries contribute to the production of testosterone only for 25% to 45% of the total synthesis,<sup>21,25-27</sup> and androgens mainly derive from precursors produced in large quantities by the adrenal cortex, that is, dehydroepiandrosterone (DHEA) and particularly DHEA sulphate (DHEAS).<sup>28</sup> In the peripheral tissues, especially in the adipose tissue, these delta5 steroids are converted into delta4 androgens, that is, androstenedione and testosterone,<sup>29,30</sup> which, in turn, through aromatization in the adipose tissue, are the source of circulating estrogens, ie, estrone (E1) and estradiol (E2).<sup>31,32</sup> 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<sup>24</sup> 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<sup>10</sup> and influences the synthesis of androgens and the activity of estrogens in peripheral tissues.<sup>31,33</sup>

## Patients and Methods

### Patients

A detailed description of the study population has been published elsewhere.<sup>24</sup> 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<sup>34</sup> 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  $-80^{\circ}\text{C}$ .

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) (Farnos, 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  $\mu\text{IU/mL}$  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  $\mu\text{g/dL}$  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<sup>35</sup> 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>).

### Statistical Methods

The hormonal variables of the women included in the study were not normally distributed and were transformed. Pearson correlation coefficients were computed between hormonal variables at baseline.

Body mass index (BMI) was computed as body weight (kg)/height (m<sup>2</sup>). The means of baseline hormone levels in the women who were treated with 1500 mg/d were compared with those of the women treated with 1000 mg/d.

The statistical analysis focused on changes in hormonal variables, calculated for each woman as the difference between values at the end of the study (ninth month) and baseline values. We used the nonparametric Wilcoxon rank sum test to compare the effect of the treatment in the women randomized to receive 1000 and 1500 mg/d of Metf. The statistical significance of the mean changes of log-transformed data in the 1500 mg/d group, compared with the 1000 mg/d group, was assessed using analysis of variance controlling for age, BMI, and weight change.

We also performed separate analyses according to baseline BMI ( $\leq 25.7$  and  $> 25.7$ , the median value of the distribution).

All of the *P* values are 2-tailed. All analyses were performed using the STATA 11 statistical package.

### Results

Ninety-six women (88.9%) completed the 9-month study, 43 women receiving 1500 mg/d, and 53 women 1000 mg/d.<sup>24</sup>

At baseline, the serum levels of testosterone, androstenedione, E1, and E2 were strongly and significantly correlated; DHEAS was significantly correlated only with androstenedione and E1. Free testosterone was strongly correlated with total testosterone, androstenedione, and E2, and less strongly with insulin and IGF1 (Table 1). Waist circumference was strongly and significantly correlated with serum insulin ( $r = 0.53$ ), E2 ( $r = 0.40$ ), E1 ( $r = 0.25$ ), and free testosterone ( $r = 0.25$ ).

The 2 groups of Metf treatment were comparable for anthropometric parameters, tumor stage, and for the proportion of nodal metastasis at diagnosis, estrogen receptor (ER)-positive tumors, and tamoxifen use, as previously reported.<sup>24</sup> At baseline, the women who took 1500 mg/d showed somewhat higher serum levels of E2 and free testosterone than the women in the 1000 mg/d group (respectively, mean  $\pm$  SD,  $7.5 \pm 8.9$  pg/mL vs.  $5.9 \pm 4.8$  pg/mL;  $P = .16$ ;  $5.30 \pm 3.12$  pg/mL vs.  $4.43 \pm 2.18$  pg/mL;  $P = .15$ ), without, however, any statistically significant differences.

Table 2 shows the comparison between baseline and ninth month values according to Metf treatment (1000 vs. 1500 mg/d); for convenience, the previously published results on insulin and testosterone are also included.<sup>24</sup>

At the ninth month of treatment, the women receiving 1500 mg/d showed a significant reduction of free testosterone ( $-29\%$ ;  $P < .01$ ) and E2 ( $-38\%$ ;  $P = .02$ ), a borderline significant reduction of IGF1 ( $P = .05$ ) and E1 ( $P = .06$ ), and a nonsignificant reduction of androstenedione ( $P = .10$ ). They also showed a nonsignificant increase of DHEAS ( $P = .25$ ). Women treated with 1500 mg/d showed significant changes also between the third and ninth month with  $P < .01$  for testosterone (total testosterone from 0.35 to 0.31 ng/mL and free testosterone from 4.6 to 3.8 pg/mL) and  $P = .03$  for E2 (from 5.5 to 4.7 pg/mL).

The women who took 1000 mg/d showed a significant increase of DHEAS ( $P = .02$ ), and a nonsignificant reduction of free testosterone and androstenedione; they showed substantially no changes in the levels of E2, E1, and IGF1. Women who received 1000 mg/d showed a nonsignificant increase in E2 and a nonsignificant reduction in testosterone (total and free) between the third and ninth month.

When comparing the 2 treatment groups (Table 2),<sup>24</sup> we found that the women who took 1500 mg/d showed a significantly greater reduction of free testosterone and E2 levels than the women treated with 1000 mg/d. The between-groups difference in the variation of free testosterone remained significant after controlling for age, baseline BMI, and weight change, and the difference in E2 remained borderline significant ( $P = .05$ ) after the adjustment. The difference in E2 lost significance after a further adjustment for change in testosterone ( $P = .17$ ).

The effect of 1500 mg/d in reducing free testosterone was significant in the normal weight women ( $n = 23$ ) and in the overweight women ( $n = 20$ ), and the reduction of E2 was significant only in the overweight women.

### Discussion

Our previous analysis showed a significant decrease in insulin, HOMA-IR index, and total testosterone levels in the women treated with Metf 1500 mg/d compared with the women who took 1000 mg/d.<sup>24</sup> Our new results suggest that the women treated with Metf 1500 mg/d also had significantly reduced serum levels of free testosterone and E2 compared with the women who took 1000 mg/d, without any modifications in DHEAS levels.

**Table 1** Pearson Correlation Coefficients on Log Transformed Data Between Baseline Hormonal Variables

Hormone	Testosterone	IGF1	Insulin	E2	E1	DHEAS	Androstenedione
IGF1	0.08	—	—	—	—	—	—
Insulin	0.10	-0.01	—	—	—	—	—
E2	0.43 <sup>a</sup>	0.05	0.23	—	—	—	—
E1	0.31 <sup>a</sup>	0.07	0.21	0.36 <sup>a</sup>	—	—	—
DHEAS	0.19	0.10 <sup>a</sup>	-0.01	0.08	0.30 <sup>a</sup>	—	—
Androstenedione	0.60 <sup>a</sup>	0.22 <sup>a</sup>	-0.03	0.33 <sup>a</sup>	0.37 <sup>a</sup>	0.29 <sup>a</sup>	—
Free Testosterone	0.77 <sup>a</sup>	0.16 <sup>a</sup>	0.17 <sup>a</sup>	0.38 <sup>a</sup>	0.22	0.16	0.46 <sup>a</sup>

Abbreviations: DHEAS = dehydroepiandrosterone sulfate; E1 = estrone; E2 = estradiol; IGF1 = insulin-like growth factor 1.

<sup>a</sup> $P < .01$  (2-sided tests).

**Table 2** Comparison Between Baseline and Ninth Month According to Treatment Group

Variable	Metformin 1000 mg/d (n = 53)		Metformin 1500 mg/d (n = 43)		P <sup>a</sup>
	Baseline	Ninth Month	Baseline	Ninth Month	
Insulin, $\mu$ IU/mL <sup>b</sup>	8.4 $\pm$ 3.8	8.3 $\pm$ 3.2	9.5 $\pm$ 5.8	7.3 $\pm$ 4.7	<.01
IGF1, ng/mL	119.7 $\pm$ 48.5	117.6 $\pm$ 43.8	114.9 $\pm$ 38.3	108.3 $\pm$ 48.6	.09
Testosterone, ng/mL <sup>b</sup>	0.36 $\pm$ 0.14	0.34 $\pm$ 0.14	0.41 $\pm$ 0.18	0.31 $\pm$ 0.13	<.01
Free Testosterone, pg/mL	4.43 $\pm$ 2.18	4.21 $\pm$ 2.19	5.30 $\pm$ 3.12	3.78 $\pm$ 2.46	<.01
DHEAS, $\mu$ g/dL	113.2 $\pm$ 67.2	125.3 $\pm$ 86.7	114.2 $\pm$ 63.3	120.3 $\pm$ 69.1	.96
Androstenedione, ng/mL	1.41 $\pm$ 0.6	1.33 $\pm$ 0.6	1.41 $\pm$ 0.7	1.27 $\pm$ 0.5	.57
E2, pg/mL	5.9 $\pm$ 4.8	5.8 $\pm$ 5.7	7.5 $\pm$ 8.9	4.7 $\pm$ 3.6	.02
E1, pg/mL	23.3 $\pm$ 8.1	23.2 $\pm$ 12.1	23.8 $\pm$ 12.6	21.7 $\pm$ 12.8	.43

Data are presented as mean  $\pm$  SD.

Abbreviations: DHEAS = dehydroepiandrosterone sulfate; E1 = estrone; E2 = estradiol; IGF1 = insulin-like growth factor 1.

<sup>a</sup>P of differences between baseline and ninth month comparing metformin 1000 mg/d vs. metformin 1500 mg/d (Wilcoxon rank sum test).

<sup>b</sup>From Campagnoli et al.<sup>24</sup>

After menopause, circulating androgens derive mainly from the adrenal gland. Adrenal androgen production decreases with age, but a fairly large quantity of androgens is produced also after menopause. DHEA, and particularly its sulphate DHEAS, is the main circulating androgen.<sup>21,28</sup> DHEAS, DHEA, and its metabolite androstenediol, are then transformed, in the adipose tissue, into delta4 androgens.<sup>29,30,36</sup> Such peripheral conversion is actually the major source of circulating androstenedione and testosterone. Approximately 15% of DHEA<sup>28</sup> and androstenedione,<sup>21</sup> and 25% to 45% of testosterone<sup>21,25-27</sup> are produced by the ovary, which after menopause maintains the ability to synthesize androgens in the stroma compartment. Ovaries are generally the origin of a relative excess of testosterone.<sup>25</sup> The reduction of testosterone levels that we observed with Metf 1500 mg/d could be either caused by an interference in the peripheral conversion of adrenal delta5 perandrogens (DHEAS and derivative) into delta4 androgens, or, more probably by the reduction of testosterone secretion by the ovaries.

The reduction of testosterone and free testosterone levels that we observed in our study could be partly explained by the decrease of insulin levels; in our previous analysis, however, the between-groups differences in the variation of testosterone levels remained significant after controlling for insulin. It is also possible that Metf directly acts on ovarian stroma in reducing testosterone production.<sup>37-39</sup> Physiological studies consistently showed that hyperinsulinemia is associated with high circulating levels of free testosterone.<sup>21,26</sup> Actually, insulin inhibits the liver synthesis of SHBG, thus increasing the bioavailability of testosterone and E2.<sup>26,40</sup> Moreover, insulin affects androgen production by increasing the activity of cytochrome P450c17 $\alpha$ , a key enzyme in the biosynthesis of androgens.<sup>39</sup> The main activity of insulin is on the ovarian and, to a lesser extent, adrenal synthesis of androstenedione and testosterone.<sup>41,42</sup> In women affected by polycystic ovary syndrome (PCOS), who usually have high levels of insulin because of insulin resistance, Metf strongly reduces testosterone, but does not substantially affect DHEAS levels.<sup>39,43</sup> Even in a randomized study on postmenopausal obese women with previous PCOS and/or insulin resistance, Metf 2000 mg/d reduced testosterone and free testosterone, and it did not modify DHEAS.<sup>25</sup> Conversely, in another recent randomized study on obese postmenopausal glucose-intolerant women, 1 year of treatment with Metf 1700 mg/d did

not reduce testosterone, and caused a nonsignificant 11% decrease in the level of DHEA.<sup>44</sup> The difference between these 2 studies for what concerns the variations of testosterone might be because of the different doses of Metf used for the treatment of obese women. It is noteworthy that, in the Kim et al study,<sup>44</sup> treatment with 1700 mg/d caused only nonsignificant reductions in insulin and glucose levels, and in the Patel et al study,<sup>25</sup> 2000 mg/d caused significant decreases in insulin (–31%) and the HOMA-IR index (–36%). In our study, which included slightly overweight women who had neither a history of previous PCOS nor of insulin resistance, in the women treated with 1500 mg/d of Metf, we observed a significant decrease in the levels of insulin (–25%), in the HOMA-IR index (–29%), and in the levels of testosterone (–23%),<sup>24</sup> and free testosterone (–29%), and no variations were observed in the women treated with 1000 mg/d. Conversely, we obtained no change in DHEAS levels.

High serum levels of testosterone increase the risk of BC, in particular ER-positive BC,<sup>18-20</sup> and the risk of BC recurrences.<sup>16,17,22,23</sup> Testosterone might directly influence tumour growth by activating androgen receptors (ARs).<sup>45,46</sup> Preclinical studies on the effects of AR activation on ER-negative BC gave controversial results, a proliferative effect being observed particularly in the cell lines expressing HER2.<sup>46-48</sup> Conversely, the bulk of preclinical studies on ER-positive BC suggests that, as physiologically happens in normal breast tissue,<sup>46</sup> testosterone has, by itself, an antiproliferative effect.<sup>46,49-52</sup> However, androgens increase the risk of ER-positive BC because of their aromatization into estrogens,<sup>31,32</sup> whose proliferative effect prevails.<sup>50,52</sup>

In our study, the women treated with Metf 1500 mg/d showed a significant reduction of E2 (–38%;  $P = .02$ ) and a borderline significant reduction of E1 (–10%;  $P = .06$ ). Consistently, in a randomized study on postmenopausal obese women with previous PCOS and/or insulin resistance<sup>25</sup> the treatment with Metf 2000 mg/d caused a significant reduction of E2 (–27%). The reduction of E2 is likely to be basically related to the decrease of testosterone—ie, the substrate for the aromatization to E2—and partially to the reduction of the adipose tissue. Furthermore, some studies suggested that Metf could directly inhibit aromatase activity.<sup>53,54</sup>

Recent studies reported that circulating estrogens are the major source of the intratumoral concentration of E2 in ER-positive

BC.<sup>32,55,56</sup> Overall, the sex hormone changes induced by Metf might have a clinical effect. Studies on Metf as adjuvant treatment in BC patients are ongoing.<sup>8</sup>

## Conclusion

We performed our trial on postmenopausal women without diabetes. We suggest that the favorable changes we observed in the sex hormone pattern of the women treated with Metf 1500 mg/d (reduction of androgens and estrogens) might be relevant also in diabetic women and might contribute to the reduction of cancer growth.

The protective effect of Metf is higher in diabetic women with HER2-positive BC, with a significant improvement of survival.<sup>5</sup> Metf might contrast HER2 activity directly or by reducing insulin levels.<sup>5</sup> A further protection might be because of the reduction of testosterone. Actually, ARs are expressed in approximately 70% of HER2-positive BCs, independent of ER status,<sup>57,58</sup> and preclinical data suggest proliferative effects of testosterone, because of cross-talk and synergy between HER2 and AR pathways.<sup>47,48</sup>

Obviously, the reduction of bioactive androgen and estrogen levels induced by Metf could be favorable in the case of ER-positive BC.<sup>16,17,22,23</sup> A possible exception, at least as far as testosterone level reduction is concerned, could be represented by women treated with aromatase inhibitors (AIs). The antiproliferative effect of testosterone on ER-positive BC prevails when estrogenic activity is low, and this could be relevant in women being treated with AIs.<sup>50,52,59</sup> However, the other major effect of Metf administration, ie, insulin reduction, is potentially favorable, because of the cross-talk between estrogen and insulin signaling pathways.<sup>7</sup>

In conclusion, our results suggest that the 1500 mg/d dose of Metf causes a significant reduction of free testosterone and E2, without any modifications of DHEAS serum levels. Because observational studies suggested that the hormonal pattern affects BC growth,<sup>16,17,22,23</sup> these changes might have prognostic importance.

## Clinical Practice Points

- Diabetic patients treated with Metf 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.
- High serum levels of insulin are associated with an increased risk of BC and BC relapses in diabetic and in nondiabetic women. Further indirect mechanisms, possibly linked to insulin reduction, could be the modifications in sex hormone bioavailability.
- High serum levels of sex hormones, androgens and estrogens, are associated with an increased risk of BC and BC recurrences. Therefore, the reduction of sex hormone bioavailability might contribute to the potential favorable effects of Metf against BC.
- This article presents the results of a further analysis of our randomized study<sup>24</sup> to test the effect of Metf on the serum levels of DHEAS, androstenedione, free testosterone, E1, and E2.
- We also evaluated the effect of Metf on IGF1, which has potent mitogenic activities and influences the synthesis of androgens and the activity of estrogens in peripheral tissues.

- The women receiving 1500 mg/d showed a greater and significant reduction of free testosterone (−29%) and E2 (−38%), a borderline significant reduction of E1 and IGF1, and a nonsignificant reduction of androstenedione. They also showed a nonsignificant increase of DHEAS.
- Metformin does not interfere with the production of DHEAS. Besides, it decreases E2 levels, basically through the reduction of testosterone. These hormonal changes might have clinical relevance.

## Acknowledgments

The study was supported by the “Assessorato alla Sanità Regione Piemonte,” by the “Fondazione CRT,” and especially by the “Compagnia di San Paolo di Torino.”

The authors thank the medical directors of the “Rete Oncologica” (Oscar Bertetto) and Sant’Anna Gynecological Hospital (Grace Rabacchi), the former directors of the Gynecologic Oncology (Giorgio Giardina) and the Endocrinologic Gynecology (Clementina Peris) Units of Sant’Anna Hospital of Torino for the support to the study, and Ms Maria Grazia Guerrini for secretarial support. The authors express their gratitude to all the patients who participated in the trial.

## Disclosure

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

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