Metformin decreases serum 8-hydroxy-2′-deoxyguanosine levels in polycystic ovary syndrome

Henri Sova, M.B.,[a,b] Ulla Puistola, M.D., Ph.D.,[b] Laure Morin-Papunen, M.D., Ph.D.,[b] and Peeter Karihtala, M.D., Ph.D.[a,b]

[a] Department of Oncology and Radiotherapy, and [b] Department of Obstetrics and Gynecology, Oulu University Hospital and University of Oulu, Oulu, Finland

Objective: To learn whether metformin treatment affects oxidative stress as measured by serum concentrations of 8-hydroxy-2′-deoxyguanosine (8-OHdG).
Design: Double-blind, randomized, placebo-controlled trial.
Setting: University outpatient clinic.
Patient(s): The study cohort consisted of 50 obese women (body mass index [BMI] ≥ 27 kg/m^2) and 60 nonobese patients (BMI <27 kg/m^2), mean age was 27.7 $\pm$ 4.0 SD years.
Intervention(s): Randomization to receive metformin or placebo for 3 months.
Main Outcome Measure(s): Serum levels of 8-OHdG before and after medical treatment.
Result(s): The levels of 8-OHdG were equal at baseline in the placebo and metformin groups. Obese women had higher baseline serum concentrations of 8-OHdG. Levels of 8-OHdG were statistically significantly reduced with metformin treatment, especially in obese patients with polycystic ovary syndrome. This study was a secondary subanalysis of a previously conducted prospective multicenter, randomized, placebo-controlled study on the effects of metformin on miscarriage, pregnancy, and miscarriage rates.
Conclusion(s): Metformin treatment, compared with placebo, statistically significantly decreased 8-OHdG levels in women with polycystic ovary syndrome.
Clinical Trial Registration Number: NCT00994812. (Fertil Steril® 2013;99:593–8. ©2013 by American Society for Reproductive Medicine.)
Key Words: 8-OHdG, metformin, oxidative stress, polycystic ovary syndrome, reactive oxygen species

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/sovah-metformin-pcos-oxidative-stress/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting approximately 5% to 10% of women of reproductive age (1). Patients with PCOS often suffer from a wide range of symptoms, including hyperinsulinemia, insulin resistance, hirsutism, amenorrhea, obesity, and infertility. Because of the heterogeneous nature of PCOS, its specific diagnosis and treatment are still problematic. It has been proposed that the pathogenesis of PCOS could be explained by alterations in insulin secretion and insulin function that could result in hyperinsulinemia and insulin resistance (2).

One of the widely used treatment modalities for PCOS is administration of metformin, which increases the uptake of glucose in peripheral tissues and inhibits gluconeogenesis. This can improve the insulin sensitivity of PCOS patients and normalize their hormone balance (1, 3). The effects of metformin are mostly brought about through activation of the 5′ adenosine monophosphate-activated protein kinase (AMPK) pathway, which is involved in the regulation of several cellular functions (3, 4). Activation of the AMPK pathway has been shown to be related to decreased glucose production, increased fatty acid oxidation, inhibition of vascular inflammation, and promotion of antioxidant defenses(3, 4).

Patients with diabetes and metabolic syndrome have significantly increased amounts of reactive oxygen species (ROS), which are primarily

Received April 11, 2012; revised September 23, 2012; accepted October 8, 2012; published online October 31, 2012.
H.S. has nothing to disclose. U.P. has nothing to disclose. L.M.-P. has nothing to disclose. P.K. has nothing to disclose.
Supported by the Finnish Medical Foundation (P.K., H.S.), the Orion-Farmos Foundation (P.K.), the North Finland Healthcare Foundation (U.P.), the Finnish Cultural Foundation (H.S.), the Medical Research Foundation of Oulu (H.S.).
Reprint requests: Henri Sova, M.B., Department of Oncology and Radiotherapy, P.O. Box 22, 90029 Oulu University Hospital, Oulu, Finland (E-mail: henrijuh@mail.student.oulu.fi).

Fertility and Sterility® Vol. 99, No. 2, February 2013 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2012.10.013

formed by physiologic processes and biochemical reactions such as oxidative phosphorylation (4–6). These extremely reactive molecules are normally restrained by antioxidant defenses. When the amount of ROS increases excessively, for example, due to overproduction or deficiency of antioxidants, then the tissues encounter a state of oxidative stress, leading to oxidative damage to cellular lipids, proteins, and DNA (7). Reactive oxygen species are linked to several pathologic conditions, including PCOS, endometriosis, cancer, ageing, diabetes neuropathy, vascular problems, schizophrenia, Alzheimer disease, and Parkinson disease (8–10). Despite their unfavorable effects, ROS also have an important role in several physiologic processes, such as fertilization, cellular signal regulation, and apoptosis (5, 10).

Direct measurement of ROS is complicated by their considerable degree of reactivity. Consequently, the best way to investigate the damage caused by ROS is to use specific oxidative stress markers. One of the most widely used oxidative stress marker is 8-hydroxy-2′-deoxyguanosine (8-OHdG), which is also considered to be the most abundant marker of oxidative damage to DNA (2). Because 8-OHdG is formed in interaction between ROS and nucleobases of DNA, its presence in DNA can lead to a loss of base-pairing specificity, misreading of adjacent pyrimidines, and insertion of adenine opposite to the lesions (11). Base excision repair (BER) is responsible for the removal of 8-OHdG from DNA, and the levels of excised 8-OHdG are easily measured in serum or urine (12).

Our study was a secondary subanalysis with a portion of patients who had been featured in a recently published prospective multicenter, randomized, placebo-controlled study on the effects of metformin on miscarriage, pregnancy, and miscarriage rates, which showed that metformin treatment compared with placebo improves pregnancy rates and live-birth rates in PCOS (13). We also found that serum 8-OHdG levels were statistically significantly lower in women with PCOS than in healthy controls, suggesting that these low levels could reflect enfeebled repair of oxidative DNA damage or enhanced antioxidant capacity rather than low ROS production in PCOS tissue. Our study further investigated the effect of metformin treatment on serum levels of 8-OHdG as a measure of oxidative stress.

MATERIALS AND METHODS

Patients

The study population consisted of 110 Caucasian women—mean age: 27.7 ± 4.0 (standard deviation [SD]) years; mean body mass index (BMI): 27.5 ± 6.2 kg/m^2—diagnosed with PCOS according to the European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) consensus definition (14). These women were sequentially selected from a large cohort of a prospective multicenter, randomized, placebo-controlled study on the effects of metformin on miscarriage, pregnancy, and miscarriage rates (13). The participants were selected from this larger pool according only to their age and BMI to meet the grouping criteria of this study. All patients were examined and recruited at the University Hospital of Oulu during 2003 to 2009. Diabetic patients, alcohol and hormone-preparation users, smokers, and women with active liver disease (alanine aminotransferase [ALAT] >2 SD above the upper normal value), past or present cardiac failure (New York Heart Association I–IV), liver or renal failure, pregnancy, or lactation were excluded.

Obese women (BMI ≥ 27 kg/m^2) received metformin (Diformin; Leiras) at 1,000 mg × 2 daily, or placebo; nonobese patients (19 kg/m^2 ≤BMI <27 kg/m^2) received metformin at 500 mg + 1,000 mg daily, or placebo. The limit for BMI was chosen on the basis of the results of prior studies that had indicated increased insulin resistance at a BMI of 27 kg/m^2 in PCOS patients (15).

All patients were evaluated 1 to 7 days after spontaneous menstruation (oligomenorrheic patients) or at any other convenient time (amenorrheic patients). The second evaluation was timed 3 months after the first visit. A total of 53 women received metformin and 57 received placebo for 3 months.

All study women had oligomenorrhea and polycystic ovaries at ultrasound. In the obese group, 22 (44%) of 50 women also had hyperandrogenism (hyperandrogenemia: serum testosterone level ≥2.3 nmol/L) and/or hirsutism (Ferriman-Gallwey score >7), as did 14 (60.8%) of 23 women in the metformin group, and 8 (29.6%) of 27 in the placebo group. In the nonobese group, 28 (46.7%) of 60 had also hyperandrogenism, as did 13 (36.9%) of 30 in the metformin group, and 15 (50%) of 30 in the placebo group. The other patient characteristics at baseline and at 3 months of treatment are shown in Table 1. This study was approved by the National Supervisory Authority for Welfare and Health (D1339/05.01.00.06/2009) and the ethics committee of the Northern Ostrobothnia Hospital District (1396/2004).

Serum Sample Assessments

Venous blood samples were taken before and after 3 months of treatment. Samples were collected and kept in polypropylene or polystyrene tubes at −20°C until the time of analysis. Serum levels of 8-OHdG were measured by means of an enzyme-linked immunosorbent assay (ELISA) using highly sensitive 8-OHdG kits from the Japan Institute for the Control of Aging (Fukuroi, Japan). The manufacturer's instructions were followed, with a few modifications, which were assay of duplicates of each sample and use of the outermost wells of the microtiter plates so that 41 samples were assayed on one plate. Any duplicate samples that differed from each other by more than 10% were reassayed (in total 9 samples out of 220). The samples from all the subgroups (metformin/placebo and BMI <and ≥ 27 kg/m^2) were randomly assessed in different ELISA batches.

Statistical Methods

We used SPSS 16.0 for Windows for statistical analyses. General linear models, Student's *t*-test, the paired samples *t*-test, and Pearson's test were used to analyze the data. Concentrations of 8-OHdG followed a normal distribution. $P<.05$ was considered statistically significant.