Effects of Short Term Metformin Treatment on Brown Adipose Tissue Activity and Plasma Irisin Levels in Women with Polycystic Ovary Syndrome: A Randomized Controlled Trial

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a chronic dysfunction associated with obesity and metabolic disorders that can be ameliorated by treatment with metformin. Brown adipose tissue (BAT) has been recently identified in adult humans, and irisin is a myokine that induces BAT formation. The aim of this randomized controlled trial was to evaluate whether a short term treatment with metformin alters BAT activity and plasma irisin levels in women with PCOS. The participants were randomly assigned to receive metformin (1500 mg/day, n = 21) or placebo (n = 24) during 60 days. BAT activity was assessed by ^{18}F-FDG positron emission tomography-computed tomography (PET-CT) and plasma irisin levels were measured by enzyme immunoassay. The groups were similar in age, body measures, metabolic profile and PCOS phenotypes. BAT activity did not change significantly in the women treated with metformin (median Δ SUV_{max} = –0.06 g/ml, interquartile interval –2.81 to 0.24 g/ml, p = 0.484, Wilcoxon's test) or placebo (median Δ SUV_{max} = 0.98 g/ml, interquartile interval –2.94 to 4.60 g/ml, p = 0.386). In addition, plasma irisin levels remained unchanged in the groups treated with metformin (median Δ = –98 ng/ml, interquartile interval –366 to 60 ng/ml, p = 0.310) and placebo (median Δ = 28 ng/ml, interquartile interval –1260 to 215 ng/ml, p = 0.650). These results suggest that in PCOS women BAT activity and plasma irisin levels may not change after a brief treatment with metformin.

Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder affecting at least one in ten adult women and characterized by androgen excess, ovulatory dysfunction and insulin resistance, which manifest as a variety of symptoms over a spectrum of clinical phenotypes [1, 2]. Although insulin resistance and glucose intolerance are not diagnostic criteria of PCOS, these features are very frequent and contribute to part of the morbidity associated with the syndrome [3].

Brown adipose tissue (BAT) is a highly energetic tissue with a unique mitochondrial expression of uncoupling protein-1 (UCP-1) that converts the cell respiration electrochemical gradient into heat, using glucose and fatty acids as substrate [4–6]. The presence of BAT in humans can be traced by positron emission tomography (PET) with ^{18}F-fluorodeoxyglucose (^{18}F-FDG), a functional imaging method that evaluates areas of high metabolic activity [7]. BAT cells are found mainly in the supraclavicular, cervical, and paraesternal regions [8] and BAT activity is reduced in older and overweight people [6, 7]. In women with PCOS, we observed lower BAT activity in the thoracic region when compare to control women of similar body mass index (BMI) but smaller waist circumference [9].

The mechanisms behind insulin resistance in women with PCOS include insulin receptor signaling defects and inhibition of insulin-mediated glucose uptake in adipocytes [10]. Metformin, a biguanide that has been used for PCOS treatment since 1994, is able to revert most of the metabolic abnormalities of PCOS [11, 12]. By increasing insulin sensitivity, metformin treatment reduces ovarian androgen production and can restore ovulation in PCOS women [12]. Another possible action mechanism of metformin could be inducing transdifferentiation of white adipocytes into BAT [13, 14] through increased production of irisin, a myokine that can induce UCP-1 activation and BAT formation [15].

Thus, the aim of this randomized, double-blind, placebo-controlled trial was to evaluate whether a short term treatment with metformin alters BAT activity and plasma irisin levels in women with PCOS.

Subjects and Methods

Study approval and registration

This randomized controlled trial (RCT) was approved by the Research Ethics Committee of Universidade Federal de Minas Gerais (Decision Number 389.782) and registered at the Brazilian Clinical Trials Registry, REBEC (Primary ID Number RBR-47tvky) and Brazilian Ministry of Health Human Research Registry (Protocol ID 17127713.2.0000.5149). All patients included in this study signed an informed consent.

Participants

The study population consisted of 45 PCOS women aged 18–45 years, enrolled from April 2015 to September 2017 at the outpatient facilities of two teaching hospitals in Belo Horizonte, Brazil. The diagnosis of PCOS was made according to the 2003 Rotterdam ESHRE/ASRM PCOS Consensus Workshop Group diagnostic criteria [16]. We excluded patients with hypo or hyperthyroidism, hyperprolactinemia, 21-hydroxylase deficiency, Cushing's syndrome and androgen producing tumors. We also excluded patients who had used anti-androgen drugs in the last 6 months or any hormones or metformin in the last 2 months, pregnant or puerperal women, women who were breastfeeding, and patients with diabetes or oral glucose intolerance.

Body measurements were performed by the same professional with the participants wearing lightweight clothing without shoes. Physical activity was assessed through the International Physical Activity Questionnaire, quantified in min/week and classified into very active, active or sedentary as detailed elsewhere [17, 18]. Women were classified as alcohol drinkers if they reported any quantity of alcohol consumption in the past month and as smokers if they smoked at least one cigarette per day [19, 20].

Brown adipose tissue activity measurement

BAT activity was measured using ^{18}F-FDG PET-CT with cold activation in the morning after 9–12 h fasting, as previously described [9]. After 1 hour resting at 19 °C, ^{18}F-FDG (0.1 mCi/kg) was administered intravenously and the subjects remained in the same cold conditions for another hour. Afterwards, a whole-body PET-CT scan was performed at 20 °C using a dedicated PET-CT system (Discovery PET-CT 600, GE Healthcare, Milwaukee, EUA). Low-dose CT without contrast enhancement was performed first and used for attenuation correction and anatomic image fusion [90 kV, 10–120 mAs (smart mA) with 3.75 mm section thickness]. The PET images were reconstructed in a 192 × 192 matrix, using an Ordered Subsets Expectation Maximization (OSEM)-like algorithm, with 2 iterations and 24 subsets.

^{18}F-FDG uptake in fat areas identified by CT was quantified using a dedicated workstation (Xeleris, GE Healthcare) [21]. BAT activity was expressed in g/ml as the maximum standardized uptake value (SUV_{max}), defined as the tissue radioactivity concentration (KBq/ml) divided by the injection dose per lean body mass (KBq/g). In each one of four body regions (cervico-thoracic, axillary, thoracic, and abdominal) a region of interest (ROI) was delimited around the most dense uptake zone and used to calculate the SUV_{max}. The highest BAT uptake value was used as the total BAT activity. All images were evaluated by two independent readers (experienced nuclear physician and radiologist), blinded to the clinical findings and laboratorial results.

Irisin assay

Blood samples were collected at 9:00 AM after an 8-hour fast between the 3rd and 5th days of menstrual cycle, and processed as described elsewhere [9]. Plasma irisin levels were assayed using a commercial ELISA kit (CUSABIO, catalog number CSB-EQ027943HU), following strictly the manufacturer's instructions, in duplicate and with the operator blinded to the patient group. The intra-assay and inter-assay coefficients of variation, using three samples of known concentration, were < 8 and < 10 %, respectively. The assay sensitivity was 0.78 ng/ml.

Intervention

The participants were randomly assigned to receive 60 days of treatment with either metformin (Glifage XR, Merck, 3 × 500 mg, single daily dose at night, n = 21), or placebo tablets (n = 24). The treatment duration and dose were chosen because a systematic review showed that 8 weeks (56 days) and 1500 mg/day were the minimum required to achieve metabolic improvement in women

with PCOS [22]. Randomization was performed through a computer generated random number list. The personnel involved in participant enrollment received sequentially numbered, opaque, sealed flasks containing the metformin or placebo tablets.

The intervention period ranged from April 2015 to September 2017 and the last follow-up assessment was in September 2017. The main outcome measure was the change in total BAT level, and a secondary outcome was the change in plasma irisin level.

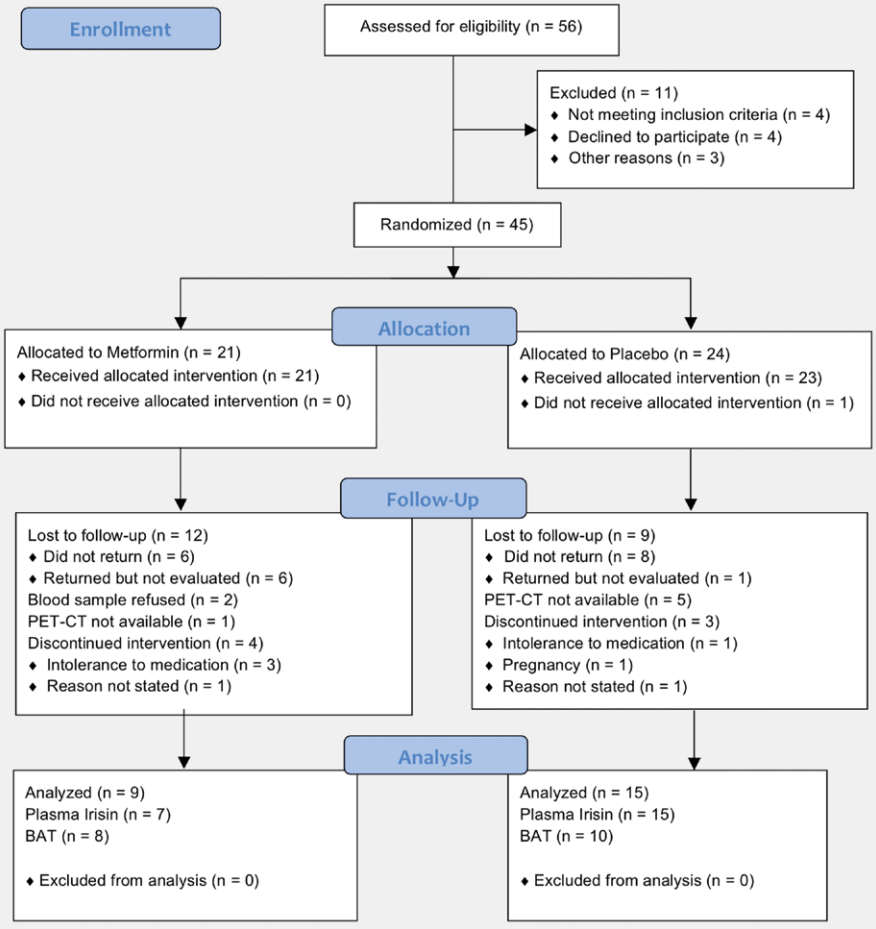
Statistical analysis

All participants available to follow-up were included in the outcome analyses regardless of adherence to the treatment protocol (modified intention-to-treat principle) [23]. Continuous variables were tested for normal distribution by Shapiro–Wilk test. Normally distributed variables were expressed as means ± SD and compared between groups using unpaired Student's *t*-test. Non-normal variables were expressed as medians with quartiles and compared by Mann–Whitney U-test (between groups) or Wilcoxon matched-pairs signed rank test (post-treatment vs. pre-treatment). Categorical variables were submitted to Chi-square test with continuity (Yates) correction or Fisher's exact test, as appropriate. All tests were two-tailed and $p < 0.05$ was considered statistically significant. Sample size calculation indicated that 21 participants per group would be sufficient to detect a minimum difference of 6g/ml in the change of BAT activity (Δ SUV_{max}) between the two treatment groups with 95 % confidence and 80 % statistical power.

▶ **Table 1** Baseline characteristics of the study participants.

Variable	Metformin (n = 21)	Placebo (n = 24)	p-Value
Age (years)	30.3 ± 4.5	30.9 ± 6.4	0.641
Weight (kg)	73.2 ± 12.7	73.5 ± 13.7	0.873
Height (cm)	161.4 ± 6.5	160.9 ± 6.0	0.707
Body mass index (kg/m^2)	28.1 ± 4.3	28.3 ± 4.9	0.900
Waist circumference (cm)	91.2 ± 10.8	90.7 ± 13.9	0.932
Blood Pressure (mmHg)			
Systolic	110 (110–120)	120 (103–120)	0.230
Diastolic	70 (70–80)	75 (63–80)	0.951
Smoking (n, %)	2 (10 %)	4 (17 %)	0.670
Alcohol drinking (n, %)	4 (19 %)	6 (25 %)	0.729
Physical activity duration (min/week)	360 (165–615)	460 (188–715)	0.495
Physical activity classification (n, %)			0.617
Very active	4 (19 %)	4 (17 %)	
Active	11 (52 %)	16 (67 %)	
Sedentary	6 (29 %)	4 (17 %)	
Current hypocaloric diet (n, %)	5 (24 %)	2 (10 %)	0.410
Serum cholesterol (mg/dl)			
Total	181.5 ± 44.6	194.5 ± 32.5	0.260
HDL-c	48.4 ± 13.9	46.7 ± 11.3	0.622
Triglycerides (mg/dl)	95 (63–133)	123 (84–167)	0.171
LAP index	41 (20–55)	53 (17–70)	0.469
Fasting glucose (mg/dl)	88.9 ± 7.9	87.7 ± 6.5	0.962
Serum insulin (µIU/ml)	13.7 ± 7.9	14.6 ± 10.7	0.724
Serum TSH (µIU/ml)	2.02 ± 0.81	3.07 ± 3.34	0.890
PCOS phenotype (n, %)			0.237
Hyperandrogenism + anovulation + PCO	15 (71 %)	11 (46 %)	
Anovulation + PCO	5 (24 %)	12 (50 %)	
Hyperandrogenism + PCO	1 (5 %)	1 (4 %)	
Infertility (n, %)	20 (95 %)	17 (74 %)	0.097
Gravidity	0 (0–1)	0 (0–1)	0.820
Previous metformin use (n, %)	10 (48 %)	6 (26 %)	0.242
Serum total testosterone (ng/dl)	49.5 ± 29.9	48.4 ± 29.6	0.606
Serum prolactin (ng/ml)	13.7 ± 4.3	13.9 ± 4.2	0.820
BAT activity (FDG uptake, SUV_{max})	7.4 (0.9–16.4)	5.2 (0.9–14.4)	0.946
Plasma irisin (ng/ml)	710 (335–1099)	950 (424–2152)	0.195

Data are expressed as means ± SD or medians (interquartile ranges). p-Values refer to unpaired *t*-test, Mann–Whitney test, Chi-square test, or Fisher's exact test, as appropriate. LAP: Lipid accumulation product; PCO: Polycystic and/or enlarged ovaries in ultrasound imaging.



▶ **Fig. 1** Flow diagram of the randomized trial of metformin versus placebo in women with PCOS.

Results

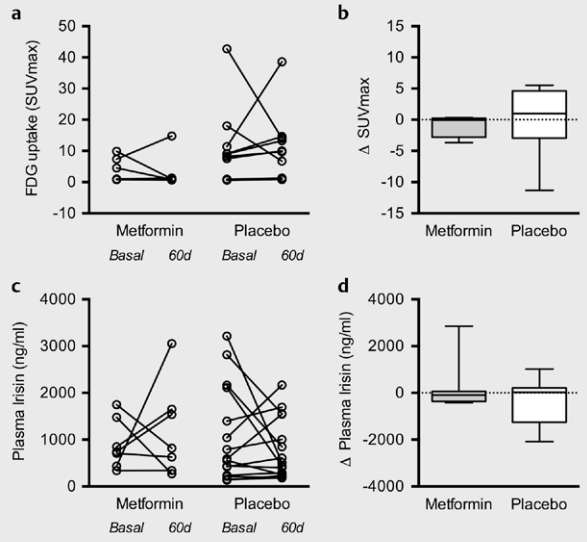
▶ **Table 1** summarizes the baseline characteristics of the two study groups. The groups were similar in age, body measures, metabolic profile and PCOS phenotypes. The trial was completed by 9 participants of the metformin arm and by 15 participants of the placebo arm (▶ **Fig. 1**). Because our PET-CT facility had a period of shutdown, 6 participants that had completed the treatment course and returned to follow-up could not be reevaluated for BAT activity. Nevertheless, the anthropometric, clinical and biochemical characteristics of the metformin and placebo groups were similar at baseline, both comparing all randomized participants (▶ **Table 1**) and comparing only those who were available for follow-up (Supplemental ▶ **Table 1S**).

As shown in ▶ **Fig. 2**, BAT activity did not change significantly after treatment with metformin (median Δ SUV_{max} = –0.06 g/ml, interquartile interval –2.81 to 0.24 g/ml, p = 0.484, Wilcoxon's test) or placebo (median Δ SUV_{max} = 0.98 g/ml, interquartile interval –2.94 to 4.60 g/ml, p = 0.386). The median change of BAT activity did not differ significantly between the metformin and placebo groups (p = 0.351, Mann–Whitney test).

Plasma irisin levels remained essentially unchanged in the groups treated with metformin (median Δ = –98 ng/ml, interquartile interval –366 to 60 ng/ml, p = 0.310) and placebo (median Δ = 28 ng/ml, interquartile interval –1260 to 215 ng/ml, p = 0.650, ▶ **Fig. 2d**), although some participants had large differences between basal and 60 day plasma irisin levels regardless of being treated with metformin or placebo (▶ **Fig. 2c**). The median change of plasma irisin levels activity did not differ significantly between the metformin and placebo groups (p = 0.702, Mann–Whitney test).

Discussion

In the present RCT, no significant change in BAT activity levels was observed from the beginning to the end of 60 days of treatment with either metformin or placebo. In a previous cross-sectional study, we had observed lower BAT activity in women with PCOS compared to healthy controls [9]. Our hypothesis was that metformin would improve insulin sensitivity and consequently rescue the normal BAT distribution in PCOS women. However, this hypothesis was not confirmed here. One possibility is that there was some improvement in insulin sensitivity with the metformin treatment but it was not sufficient to induce measurable changes in BAT activity. Early "browning" adipocytes (known as beige or brite) may not be evidenced by PET-CT since the cells that undergo the transdifferentiation process are immersed and dispersed in white fat sites [24]. In this context, high-resolution imaging coupled with



▶ **Fig. 2** Individual plots (**a**, **c**) and median variations (**b**, **d**) of BAT activity and plasma irisin levels in PCOS women treated with metformin or placebo. The graphs represent the difference (Δ) between measures obtained before and after 60 days of treatment.

molecular characterization of tissue specimens may be needed to localize this early type of BAT [25]. In addition, we excluded women with glucose intolerance or diabetes, who are expected to benefit most from the use of metformin.

While the present study addressed the hypothesis that an insulin sensitizer (metformin) might increase BAT activity in humans, preclinical evidence suggests that interventions to increase the amount of BAT ultimately improve insulin sensitivity. Hu et al. [26] observed that three weeks of treatment with rutin, a novel compound for BAT activation, improved thermogenesis and systemic insulin sensitivity in a rat model of androgen-induced PCOS. Interestingly, BAT transplantation increased serum adiponectin and remarkably improved glucose homeostasis and insulin sensitivity in PCOS-like rats [27]. These translational results suggest that BAT activity is related to PCOS complications and that BAT induction might be a therapeutic option for PCOS treatment.

In this study, we observed no effect of metformin treatment for 60 days on plasma irisin levels in PCOS women. Our previous observations [9] as well as an extensive meta-analysis [28] indicated that plasma irisin levels are not altered in women with PCOS when compared with healthy controls of similar BMI. We decided to measure plasma irisin levels in the present study because irisin promotes UCP-1 expression and BAT differentiation with consequent improvement in glucose metabolism [15], therefore irisin could mediate any effect of metformin on BAT formation.

One possible reason for the lack of change in plasma irisin might be the relatively short treatment time, as a previous single-arm study of metformin treatment in PCOS women found a decrease in circulating irisin levels only at 6 months follow-up [29]. Another previous trial without a control treatment group administered metformin at low dose (500 mg once daily) during 3 months and observed a decrease of about 20 % in plasma irisin levels [30]. These studies were open-label and had no control group, carrying the risk of performance and detection biases. Our study had a double-blind, placebo-controlled design but it had a shorter intervention time and many participants were lost to follow-up. Therefore, further evidence from RCTs is still needed to clarify with a higher degree of confidence whether metformin treatment is able to change plasma irisin levels in women with PCOS.

As far as we know, this study is the first to evaluate the effect of metformin on BAT activity in humans. Our study achieved homogeneity between the metformin and placebo groups and was double blind to avoid the risk of selection, performance and detection biases. However, an important limitation of the present study is the smaller sample size at the end of the trial as a result of the participants lost to follow-up. While attrition bias is not evident by comparing the baseline characteristics of the participants that were fully analyzed, the statistical power of the trial was reduced. Consequently, further studies with a larger population are warranted.

In conclusion, no change in BAT activity and plasma irisin levels was observed after a brief treatment with metformin in women with PCOS.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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