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DPP4 Inhibitor Sitagliptin as a Potential Treatment Option in Metformin Intolerant Obese Women with Polycystic Ovary Syndrome: a Pilot Randomized Study

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Abstract

Objective: Metformin has an established role in the management of polycystic ovary syndrome (PCOS). Some patients cannot tolerate it due to associated gastrointestinal adverse events. The present study evaluated DPP4 inhibitor sitagliptin as a potential treatment option in metformin intolerant PCOS.

Design: We conducted a 12-week prospective randomized open-label study with 30 obese metformin intolerant women with PCOS (aged 35.0±7.2 years, BMI 36.9±5.5 kg/m2). After metformin withdrawal they were randomized to lifestyle intervention and sitagliptin 100mg QD (SITA) or lifestyle intervention alone as controls (CON).

Methods: All participants underwent anthropometric and endocrine measurements and OGTT. Model derived indexes of insulin resistance (IR) and beta-cell function were calculated.

Results: SITA improved beta-cell function as assessed by homeostasis model assessment for beta-cell function index (HOMA-B) for 45.9±35.8 (p=0.001), modified beta-cell function index (MBCI) for 7.9±7 (p=0.002) and quantitative insulin sensitivity check index (QUICKI) for -0.03±0.03 (p=0.002). By contrast, beta-cell function decreased in CON. The between group differences were significant for HOMA-B (p=0.000), MBCI (p=0.010) and QUICKI (p=0.025). The conversion rate to impaired glucose homeostasis was prevented in SITA: 3/15 of subjects had IGT before and after the study. In CON none had T2D and 4 had IGT at the beginning. After 12 weeks IGT was observed in 2 and T2D in 3 subjects.

Conclusion: SITA improved beta-cell function and prevented a conversion rate to IGT and T2D in metformin intolerant obese PCOS.

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Abbreviations:
BMI = body mass index; **DHEAS =** dehydroepiandrosterone sulphate; **DPP4 =** dipeptidyl peptidase-4; **DXA =** Dual Energy X-ray Absorptiometer; **GIP =** glucose-dependent insulinotropic peptide; **GLP-1 =** glucagon-like peptide-1; **HOMA-B =** homeostasis model assessment for beta-cell function ; **HOMA-IR** = homeostasis model assessment of insulin resistance; **IAI =** insulin action index; **IGT =** impaired glucose tolerance; **IR =** insulin resistance; **MBCI =** modified beta-cell function index; **OGTT =** oral glucose tolerance test; **QUICKI =** quantitative insulin sensitivity check index; **PCOS =** polycystic ovary syndrome; **SHBG =** sex hormone-binding globulin; **T2D =** type 2 diabetes; **VAT =** visceral adipose tissue.

Introduction

Metformin has an established role in the management of PCOS with high metabolic risk (1). It is undoubtedly effective in reducing the rate of conversion to type 2 diabetes (T2D) (2). In addition, several studies have suggested its positive effect on menstrual irregularities irrespective of pre-treatment presence of insulin resistance (IR) or weight (3-5).

Its long-term safety profile is favorable, but up to 25% of patients suffer metformin-associated gastrointestinal effects (6)**.** They could have a deleterious effect on quality of life and result in high rates of non-adherence (7). Tolerance is affected by genetic variation in intestinal transporters of metformin, the production of lactate concentrations within the enterocyte, increases in glucagon-like peptide-1 (GLP-1) concentrations, bile acid pool and altered microbiome (8)**.** The strategies to lessen the adverse events include appropriate titration of immediate release metformin, use of extended release metformin and gut microbiome modulators. However, approximately 5% of patients are still unable to tolerate metformin at all (6)**.**

One of the major clinical problem that appear in metformin intolerant PCOS subjects is how to reduce the progression rate of normal glucose tolerance to impaired glucose tolerance (IGT) and in turn to T2D. The progression rate in metabolically unfavorable phenotypes of PCOS may be as high as 5 to

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15% within 3 years (9, 10). More than 2% of untreated PCOS women have a risk of developing T2D each year (11). Body mass index (BMI), visceral adiposity and weight gain were found as strong predictors of progression in glucose derangements. In addition, insulin resistance (IR) and beta-cell dysfunction were also recognized as underlying important risk factors for the development of T2D in PCOS (11). Only few studies have addressed preservation of beta-cell function as a potential treatment target in this population (12, 13).

Dipeptidyl peptidase-4 (DPP-4) inhibitors can halt or reverse the loss of functional beta-cell mass in animal models and may delay transition to pre-diabetes and T2D (14-16). DPP-4 inhibition as an alternative therapeutic option had been proposed yet untested in metformin intolerant women with PCOS. The present study is the first investigation into the role of DPP-4 inhibitors in metformin intolerant obese PCOS. The aim was to evaluate whether DPP-4 inhibitor sitagliptin in combination with lifestyle intervention preserves beta-cell function in this population. The secondary outcomes were changes in other measures of glycemic control including conversion rates to IGT and T2D, changes in hormonal parameters, anthropometric measures of obesity and basal levels of incretin hormones.

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Subjects and methods

Study design

We conducted a 12-week prospective randomized open-label study with 30 obese women with PCOS aged 35.0±7.2 years with BMI 36.9±5.5 kg/m^2 that had been intolerant to metformin. They were recruited in July and August 2016 from the outpatients Department for Endocrinology, Diabetes and Metabolic Diseases University Medical Center Ljubljana. Before they were defined as metformin intolerant they were on metformin up to 8 weeks. Metformin intolerance was defined with the presence of intolerable gastrointestinal adverse events encountered with metformin before randomization include diarrhea, nausea, flatulence, indigestion, vomiting and abdominal discomfort. After metformin intolerance was reported by the patients the washout period before randomization was from 1 up to 2 weeks.

All included subjects fulfilled Rotterdam criteria with metabolically unfavorable type A phenotype of PCOS including concomitant presence of a) hyperandrogenemia on either the biochemical or the clinical level, b) menses abnormalities and c) PCO morphology (17). All of them had normal serum prolactin concentrations and thyroid function tests. Possible Cushing's syndrome was excluded when clinically indicated. When basic 17α-hydroxy-progesterone was > 6.1 nmol/L (2 ng/ml) the determination of 17α-hydroxy-progesterone after stimulation with Synacthen test was performed to rule out non-classic congenital adrenal hyperplasia.

They were eligible for enrollment if they were aged 18 years to menopause and obese (BMI ≥ 30). Exclusion criteria were significant kidney or hepatic disease, acute and chronic pancreatitis or known history of pancreatitis or gallbladder disease. Pregnancy was excluded by measuring beta-human chorionic gonadotropin.

At the beginning of the study lifestyle intervention was reinforced in all subjects. They received individual counseling regarding lifestyle Intervention. Reducing diet of 500-800 kcal/day reduction made up of 50% carbohydrates preferably with low glycemic index, 20% proteins and 30% of fat

DOI:10.4158/EP-2017-0027

mostly mono- and poly- unsaturated, with the amount of saturated fat less than 10%. The participants were encouraged to increase consumption of fiber, whole grains, cereals, fruits and vegetables along with at least 30 min of moderate intensity physical activity daily was promoted. Type of physical activity has been chosen on the basis of ability and compliance for individual patient, most of them performed walking or cycling.

All participants were asked to record all food eaten and physical activity made daily, and to self-report about their compliance in 4th in 8th week of the study, when they were seen in person or contacted by phone. They had additional counseling via phone in order to promote adherence to the diet and exercise when needed.

Women were randomized to lifestyle intervention and sitagliptin (SITA) or lifestyle intervention alone as controls (CON). Post randomization and at the end of the study all participants underwent standard anthropometric measurements: height, weight, waist circumference. Assesment of whole-body composition was measured by a Hologic Dual Energy X-ray Absorptiometer (DXA), including visceral adipose tissue (VAT) area. A fasting blood sample was drawn for determination of glucose, insulin, C-peptide, total GLP-1, glucose-dependent insulinotropic peptide (GIP), total and free testosterone (T), androstenedione, dehydroepiandrosterone sulphate (DHEA) and sex hormone-binding globulin (SHBG), followed by a 2-h oral glucose tolerant test (OGTT). Blood samples for glucose were drawn at 0, 60 and 120 min of OGTT. According to American Diabetes Association (ADA) guidelines OGTT was used to screen for IGT and T2D (18). History of menstrual pattern was recorded. Safety clinical assessment was performed at the beginning and at the end of the treatment period. Lifestyle intervention was monitored and supported by offering individual consultations at baseline and at the 4th and the 8th week of the study.

The study was approved by a Slovene National Medical Ethics Committee and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was obtained from all patients before participation. The study is registered on www.ClinicalTrials.gov as NCT03122041.

DOI:10.4158/EP-2017-0027

Biocemical assays

Glucose levels were determined using a standard glucose oxidase method (Beckman Coulter Glucose Analyzer, Beckman Coulter Inc CA, USA). Insulin was determined by immunoradiometric assay (Biosource Europe S.A., Nivelles, Belgium). C-peptide was measured using an immunoenzymometric method based on specific monoclonal antibodies.

Total GLP-1 levels were measured in plasma using commercially available kit Total GLP-1 ELISA (7-36 and 9-36) (Alpco, 43-GPTHU-E01), according to the manufacturer's instructions. Briefly, standards, controls and samples were added in duplicates to the designated wells of the streptavidin coated microplate, followed by total GLP-1 tracer antibody and total GLP-1 capture antibody mixture. The plate was covered and incubated for 20-24 hours at 2-8 OC. On the next day, the plate was washed and substrate was added into each well. The plate was incubated in the dark for 20 minutes at room temperature. Stop solution was added and the absorbance was read at 450nm/620nm within 10 minutes, using Tecan Safire (Tecan Group Ltd., Switzerland).

Total GIP levels were measured using Human GIP (Total) ELISA kit (EZHGIP-54K, EMD Millipore, USA), according to the manufacturer's instructions. Briefly, assay buffer was added to the microplate, coated with anti-GIP monoclonal antibodies, followed by the matrix solution to the blank, standard and control wells. Standards, controls and samples were added to the designated wells in duplicates. The plate was incubated for 1.5 hours at room temperature. After washing, the detection antibodies were added to all the wells and the plate was incubated for 1 hour at room temperature, followed by washing, addition of enzyme solution, and subsequent 30 minutes incubation. The plate was washed and substrate solution was added for 20 minutes, after which the reaction was stopped and absorbance was read at 450nm/590nm within 5 minutes, using Tecan Safire (Tecan Group Ltd., Switzerland).

Total and free testosterone levels were measured by coated tube RIA (DiaSorin, S. p. A, Salluggia, Italy and Diagnostic Products Corporation, LA, respectively). Androstenedione and DHEAS were measured by specific double antibody RIA using 125 I-labeled hormones (Diagnostic Systems Laboratories, Webster, Tx). Sex hormone binding globulin (SHBG) was determined with a chemiluminescent immunoassay (Immulite 2000 Analyzer, Siemens Healthcare, Erlangen, Germany).

DOI:10.4158/EP-2017-0027

Pre- and posttreatment samples from each patient were assayed in the same assay run.

Assessment of human body composition by Dual Energy X-ray Absorptiometry

Whole-body composition was assessed by a DXA (Discovery A; Hologic, Waltham, MA, USA) with the software provided by the manufacturer (QDR for Windows Version 12.5).

Calculations

Body mass index **(**BMI) was calculated as the weight in kilograms divided by square of height in meters.

To assess beta-cell function basic insulin secretion function index (HOMA-B), modified beta-cell function index (MBCI) (19) and quantitative insulin sensitivity check index (QUICKI) (20) was calculated.

As a measure of insulin resistance (IR) homeostasis model assessment (HOMA-IR) calculation (21) and insulin action index (IAI) was applied. (19)

The respective formulas for calculating the above mentioned parameters were as follows: HOMA-B = 20× I0/(G0-3.5), MBCI = I0×G0/(G120+G60-7), QUICKI = 1/[log(I0) + log(G0)]), HOMA-IR=I0× G0/22.5 and IAI = 1 /(I0×G0) wherein I0 (mU/L) denotes fasting plasma insulin, G0 (mmol/L) fasting plasma glucose, G60 (mmol/L) plasma glucose level at 60 minutes after glucose load, and G120 (mmol/L) plasma glucose level at 120 minutes after glucose load in OGTT test.

Statistical analysis

Sample size was determined based on data for mean change of HOMA-B from previous studies with comparative treatment intervention using Power and Sample Size Calculation version 3.0.43 (Dupont, 1990). To detect a statistically significant difference of approximately 65 in change of HOMA-B between groups with 80% power (α=0.05), each group had to consist of 14 patients.

Mean values with standard deviations were used to describe continuous variables. To compare pretreatment and post-treatment values of continuous variables, we used nonparametric Wilcoxon signed-rank test for related samples, while nonparametric Mann-Whitney test was used to compare the change of clinical parameters among different treatment groups. Two tailed P values of <0.05 were

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considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics version 19.0 (IBM Corporation, Armonk, NY, USA)

DOI:10.4158/EP-2017-0027

Results

Baseline results

The study enrolled 30 participants. Two patients in CON discontinued the study because of protocol violation. 15 women in SITA and 13 in CON completed the study. Baseline characteristics of the patients are provided in Table 1. There were no statistically significant differences between both groups at baseline.

Static measures of beta-cell function

SITA significantly improves fasting measures of beta-cell function including HOMA-B, MBCI and QUICKI. By contrast, HOMA-B significantly decreased whereas MBCI and QUICKI did not change in CON (Table 1). The between treatment differences in static measures of beta-cell function were statistically significant for HOMA-B, MBCI and QUICKI (Table 2)

Changes in other measures of glycemic control

In CON fasting glucose was significantly increased after 12 weeks (Table 1). Glucose after 120 and 180 min increased in CON at the end of the study, while glucose at 180 min decreased in SITA, yet the within and between treatment differences were not statistically significant yet (Table 1 and 2).

Fasting insulin and C peptide increased significantly in SITA and did not change significantly in CON (Table 1). The between treatment differences for both parameters were statistically significant (Table2).

Due to significant increase in fasting insulin HOMA-IR significantly increased and IAI significantly decreased in SITA (Table 1). The increase in HOMA-IR and decrease in IAI was significant when compared to CON (Table 2).

Glucose tolerance state as assessed by OGTT

The conversion rate from normal glucose tolerance to IGT or T2D was prevented in SITA. Three subjects had IGT before and after the study and no one had T2D. In CON 4 women had IGT at the beginning. No one had T2D. After 12 weeks IGT was observed in 2 and T2D in 3 subjects in CON.

DOI:10.4158/EP-2017-0027

Secondary outcomes

Measures of obesity

After metformin withdrawal subjects shifted to lifestyle intervention in CON regain on average 3.4 ± 4.5 kg (p=0.007) compared with 2.1 ± 3.9 kg weight regain in SITA group (p=0.06). Waist circumference and VAT mass, volume and area as measured by DXA significantly increased in CON compared to statistically insignificant increase in SITA (Table 1). The between treatment differences for measures of obesity were not statistically significant yet, but there was a tendency that sitagliptin in combination with lifestyle intervention had been superior in preventing weight regain after metfromin withdrawal when compared to lifestyle intervention alone (Table 2).

Incretin hormones

Total baseline GLP-1 and GIP concentrations insignificantly increase in SITA, whereas they significantly decrease in CON (Table1). The between group difference was statistically significant for GLP-1 (Table 2).

Endocrine outcomes

After metformin withdrawal within 12 weeks of the study follow up period total and free testosterone increased significantly in CON. IN SITA no significant changes were observed in androgen profile at the end of the study as compared to baseline values (Table 1). There was statistically significant difference between groups in free testosterone, SITA being superior to CON in preserving androgen profile after metformin cessation (Table 2).

Menstrual pattern

No statistically significant changes were found, neither over time nor when analysing it separately by intervention arm (Table 1 and 2).

Adverse events

Reporting of all transient or intermittent adverse events and precise duration periods were incorporated in our study protocol. Headache was documented once in SITA. In CON group 1 woman found hard to adhere to the recommended lifestyle measurements and needed additional counseling. No severe adverse reactions were observed in either groups.

DOI:10.4158/EP-2017-0027

DOI:10.4158/EP-2017-0027

Discussion

We provided the first evidence to date that DPP-4 inhibition with sitagliptin in adjunct to lifestyle intervention improved beta-cell function and prevented a conversion rate to IGT and T2D in metformin intolerant obese women with PCOS, whereas lifestyle intervention alone resulted in more deleterious. glucose homeostasis. In addition, sitagliptin prevented significant weight regain as well as increase of visceral adipose tissue. Concentrations of incretin hormones tended to increase in SITA, whereas they significantly decrease in CON. We also demonstrated that SITA was superior to lifestyle intervention alone in preserving androgen profile.

Attempts to preserve and restore beta-cell function are important to decrease conversion rates to T2D in populations with high metabolic risk (22). The Diabetes Prevention Program (DPP) and several other studies showed that implementing a program that decreased body weight through diet and exercise reduced the incidence of T2D in patients with IGT (23). The effect of lifestyle intervention on reducing the incidence of T2D is related to overall improvements in beta-cell function driven by its gains in insulin sensitivity (24). Physical activity per se has a beneficial effect on insulin sensitivity in normal as well as insulin resistant populations (25, 26). It influences insulin sensitivity trough decreased insulin resistance and beta-cell load accompanied to weight reduction and through direct influence on GLUT4 and muscle uptake of glucose (25, 27). Beneficial effect of physical activity on insulin sensitivity indexes were also demonstrated in patients with PCOS (28).

However, metformin intolerant obese PCOS are not expected to adequately respond to lifestyle modification after metformin withdrawal since they had been unsuccessful even before they had been assigned to metformin.

We demonstrated in the present study that lifestyle intervention in women that had been withdrawn from metformin due to its intolerance resulted in worsening of beta-cell function and in a tendency of increasing IR mainly driven by a significant increase in fasting glucose levels. We noticed progression of glucose homeostasis's impairments toward T2D in 3 subjects in a very short period of follow up.

By contrast, we observed significant improvement in beta-cell function when metformin intolerant obese women with PCOS were switched to DPP-4 inhibitor sitagliptin in adjunct to lifestyle

DOI:10.4158/EP-2017-0027

intervention. Unexpectedly, as oppose to insignificant increase in controls, insulin resistance as assessed by HOMA-IR in sitagliptin patients increased significantly. The increase in both arms was most probably related to metformin withdrawal and weight gain. Since DPP4-inhibitors do not increase IR, more pronounced increase in HOMA-IR with sitagliptin when compared to controls, does not have mechanistically well-suited explanation. It could be related to the discussed limitations of the study including small number and the chosen static models for assessing IR. Importantly, the beneficial impact of sitagliptin in our study was proven in other clinically relevant measures of glucose homeostasis. Rapid deteriorations of glucose homeostasis was prevented with sitagliptin. Beneficial observations were in line with the present evidences suggesting that DPP-4 inhibitors improve beta-cell function in humans based on both static and dynamic parameters. (13, 29).

Furthermore, we demonstrated significantly differential effects of the two interventions regarding basal levels of incretin hormones. Total fasting GLP-1 and GIP concentrations insignificantly increase in SITA, whereas they significantly decrease in CON from baseline to the study end. The observations were consistent with the present evidences. It was demonstrated before that metformin could increase GIP and GLP-1 by increasing GLP-1 biosynthesis and secretion and also by inhibiting GLP-1 degradation by inhibiting DPP-4 (30). Therefore, it was expected that both incretin hormones would significantly decrease in CON after metformin withdrawal and due to weight regain in CON group. On the other hand, it was expected that sitagliptin would further increase fasting levels of incretins after metformin cessation since it was demonstrated before that DPP-4 inhibition increases not only prandial but also fasting levels of GLP-1 and results in an overall increase in GLP-1 levels with maintenance of circadian rhythm throughout the day (31).

In addition to beneficial effects on beta-cell function, glucose tolerance state and levels of incretin hormones, sitagliptin also assisted with maintenance of body weight after metformin withdrawal, whereas a shift to lifestyle intervention alone after metformin withdrawal surprisingly resulted in significant weight regain in particular due to increase in visceral adiposity. The observations imply that metformin had provided a favorable weight neutral homeostasis that could be related with its demonstrated stimulatory effect of GLP-1 (30, 32-35). Similarly, it is well established that DPP-4 inhibitors assist in favorable weight maintenances (36). A potential mechanism regarding favorable weight neutrality of DPP4 inhibitors includes moderately delayed gastric emptying via enhancement of

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GLP-1 axis (37), inhibitory effect on fat absorption from the gut and promotion of lipolysis in adipose tissue in the postprandial state, in conjunction with increased fatty acid oxidation in skeletal muscle(38).

Furthermore, SITA was superior to CON in preserving androgen profile after metformin cessation, whereas testosterone increased in controls. The study was not design to clarify whether this effect is coincidental or associated with differential effects of two interventions on glucose homeostasis, body weight and body composition. The known effect of metformin on androgen production has been controversial. Some assigned the improvement in androgen profile to the reduction of insulin resistance. It may also be argued that metformin effect on androgens could be a byproduct of ovulation resumption. In vitro studies demonstrated that metformin inhibited androgen production by theca cells. It has also been suggested that metformin reduces pituitary luteinizing hormone and increases the production of SHBG (39).

The changes in menstrual frequencies in either group were not statistically significant, most probably due to the short duration of the study.

The results of our study are subject to several limitations. An assessment of the impact of sitagliptin in adjunct to lifestyle intervention on improved beta-cell function and on the conversion rate to IGT and T2D is limited due to short observational period. However, significantly differential effects between the arms that was observed even in that short follow up period underlies the potential role of sitagliptin in adjuct to lifestyle intervention in metformin intolerant women with PCOS. Furthermore, we performed merely static measures of beta-cell function and IR while dynamic measures were not addressed. However, any tests performed for estimating beta-cell function and IR have limitations and should be regarded as mere surrogate estimates. We exposed the parameters from the equation that mainly drove the change in HOMA indexes to highlight the differences between two arms not being obvious from the results of the changes in composite calculations. An important add on value to the mere estimation of beta-cell function was that we interpreted the improvement of beta-cell function in the context of other measures of glucose homeostasis and changes in measures of obesity.

The main strength of this study is the first insight into the potential role of DPP-4 inhibition in metformin intolerant PCOS. Although the small sample size and short duration limited the generalizability of our

DOI:10.4158/EP-2017-0027

results, we had provided several evidences implying that sitagliptin as an add on to lifestyle intervention might have beneficial effects on glucose tolerance state, weight maintenance, body composition and even androgen profile in metformin intolerant PCOS when compared to lifestyle alone.

In conclusion, the high prevalence of conversion rate in obese PCOS with phenotype A suggests that the normal time-related ontogeny of IR to T2D has been subverted in this population. The progression rate from initial compensation through excess beta-cell secretion of insulin through an eventual time related decline in insulin secretion followed by the development of fasting hyperglycemia and glucose intolerance is significantly increased without intensive intervention. DPP-4 inhibitors seem to be a promising alternative in PCOS women with high metabolic risk that have failed with lifestyle intervention and are metformin intolerant. Future larger designs of longer duration should be powered using our preliminary results.

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Declaration of interest

The authors declare there is no conflict of interest in this work and have no financial interest to disclose.

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Author contributions

MJ and AJ designed the study. SF and MJ conducted the study. SF was involved in collecting the data. All authors helped writing the manuscript and have read and approved the final version.

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Tables

Table legend:

Table 1: Pretreatment and post treatment values of clinical parameters of PCOS patients for each of the treatment groups expressed as mean ± SD.

Table 2: Comparison of absolute change in clinical parameters of PCOS patients among different treatment groups compared with nonparametric Mann-Whitney test.

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Table 1: Pretreatment and post treatment values of clinical parameters of PCOS patients for each of the treatment groups expressed as mean ± SD.

	SITAGLIPTIN N=15			LIFESTYLE N=13			Comparison of pretreatment values between groups
Characteristic	Pretreatment	Post treatment	P	Pretreatment	Post treatment	P	P
Age (years)	35.1 ± 6.4			34.8 ± 8.2			0.821
BMI (kg/m^2)	37 ± 6.2	37.8 ± 5.9	0.050	36.8 ± 4.9	38 ± 5	0.008	0.650
HOMA-B	141.7 ± 56.2	187.6 ± 69.4	0.001	173.3 ± 60.2	136.9 ± 70.1	0.001	0.370
MBCI	7.9 ± 3.9	15.8 ± 8.6	0.002	8.4 ± 5.4	10.5 ± 10.1	0.507	0.964
QUICKI	0.35 ± 0.04	0.32 ± 0.03	0.002	0.35 ± 0.04	0.35 ± 0.04	0.591	0.856
Glu 0 min OGTT (mmol/l)	5.2 ± 0.5	5.5 ± 0.4	0.082	4.9 ± 0.5	5.5 ± 0.9	0.009	0.072
Glu 30min OGTT (mmol/l)	8 ± 1	8.1 ± 1.9	0.851	8.7 ± 1.6	8.3 ± 2.9	0.889	0.363
Glu 60min OGTT (mmol/l)	7.9 ± 1.8	7.4 ± 2.4	0.167	9 ± 2.3	9.1 ± 3.4	0.937	0.201
Glu 120min OGTT (mmol/l)	6.8 ± 1.3	7 ± 1.8	0.531	6.9 ± 1.9	7.7 ± 3	0.185	0.892
Insulin (mU/l)	10.8 ± 4.8	18.3 ± 8.7	0.002	13.4 ± 7.8	14.3 ± 9.6	0.506	0.555
C-peptide (pmol/l)	0.9 ± 0.3	1.3 ± 0.5	0.003	1 ± 0.4	1 ± 0.5	0.281	0.717
HOMA-IR	2.7 ± 1.3	4.5 ± 2.3	0.002	3.1 ± 2.2	3.7 ± 2.8	0.152	1.000
IAI	0.02 ± 0.02	0.01 ± 0.01	0.003	0.02 ± 0.01	0.02 ± 0.02	0.600	0.751
Weight (kg)	101.4 ± 14	103.5 ± 12.7	0.056	97.8 ± 8.3	101.2 ± 8.6	0.007	0.856
Waist circumference (cm)	105.5 ± 12.2	106.7 ± 13.5	0.061	106.8 ± 8.4	109.8 ± 9.7	0.005	0.683
DXA %fat	45.4 ± 6.2	45.2 ± 5.7	0.649	45.5 ± 4.7 [1]	45.8 ± 4.5	0.753	0.867
VAT mass (g)	763.9 ± 257.7	787.5 ± 276.3	0.460	728.6 ± 264.9 [1]	810 ± 264.7	0.015	0.683
VAT volume (cm^3)	825.7 ± 278.5	851.2 ± 298.8	0.460	787.6 ± 286.5 [1]	878.2 ± 288.7	0.012	0.683
VAT area (cm^2)	158.4 ± 53.4	163.3 ± 57.3	0.460	151.2 ± 55 [1]	168 ± 54.9	0.018	0.683
GLP-1 (pM)	4.9 ± 1.8	5.2 ± 2.3	0.281	5.4 ± 3.5 [1]	4.8 ± 2.8 [1]	0.016	0.456
GIP (pg/ml)	58.8 ± 24.3	63.1 ± 38.6	0.683	66.8 ± 35.8 [2]	54 ± 38.9 [1]	0.059	0.721
Free testosterone (pmol/l)	4.6 ± 2.8	4.7 ± 2.1	0.753	2.9 ± 1.7	6.8 ± 3.7	0.011	0.108
Total testosterone (nmol/l)	1.7 ± 1.8	1.7 ± 1.8	0.449	1.2 ± 0.5	1.5 ± 0.8	0.012	0.892
Androstenedione (nmol/l)	4.1 ± 1.5	4.5 ± 1.8	0.394	4.8 ± 2.6	4.9 ± 1.9	0.944	0.586
DHEAS (µmol/l)	5.8 ± 2.9	5.8 ± 3.3	0.842	5.6 ± 2.8	6 ± 2.9	0.278	0.856
SHBG (nmol/l)	29.1 ± 25.3	22.5 ± 7.9	0.362	62.5 ± 86.3	54.3 ± 61 [1]	0.422	0.316
Number of periods in 3 months	2.5 ± 0.7	2.3 ± 0.9	0.414	2.8 ± 0.4	2.7 ± 0.7	0.705	0.316

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Table 2: Comparison of absolute change in clinical parameters of PCOS patients among different treatment groups compared with nonparametric Mann-Whitney test.

	SITAGLIPTIN	LIFESTYLE	P

Abbreviations: BMI, body mass index; HOMA-B, homeostasis model assessment for beta-cell function; MBCI, modified beta-cell function index; QUICKI, quantitative insulin sensitivity check index; Glu, glucose; OGTT, oral glucose tolerance test; HOMA-IR, homeostasis model assessment of insulin resistance; IAI insulin action index; DXA Dual Energy X-ray Absorptiometer; VAT visceral adipose tissue; GLP-1 glucagon-like peptide-1; GIP glucose-dependent insulinotropic peptide; DHEAS dehydroepiandrosterone sulphate SHBG, sex hormone-binding globulin.

For each parameter, missing data are presented in brackets []

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Characteristic	Absolute change (mean ± SD)	Absolute change (mean ± SD)	
BMI (kg/m^2)	0.8 ± 1.4	1.3 ± 1.7	0.751
HOMA-B	45.9 ± 35.8	-94.8 ± 83.7	0.000
MBCI	7.9 ± 7	2.1 ± 7	0.010
QUICKI	-0.03 ± 0.03	0 ± 0.04	0.025
Glu 0 min OGTT (mmol/l)	0.3 ± 0.6	0.7 ± 0.9	0.156
Glu 30 min OGTT (mmol/l)	0.1 ± 1.7	-0.4 ± 2.6	1.000
Glu 60 min OGTT (mmol/l)	-0.5 ± 1.2	0.1 ± 2	0.555
Glu 120 min OGTT (mmol/l)	0.2 ± 0.9	0.8 ± 1.8	0.496
Insulin (mU/l)	7.5 ± 6.7	0.9 ± 3.5	0.005
C-peptide (pmol/l)	0.4 ± 0.4	0.1 ± 0.2	0.017
HOMA-IR	1.8 ± 1.7	0.6 ± 1.3	0.046
IAI	-0.01 ± 0.01	0 ± 0.01	0.013
Weight (kg)	2.1 ± 3.9	3.4 ± 4.5	0.717
Waist circumference (cm)	1.2 ± 2.7	3.0 ±2.5	0.108
DXA %fat	-0.2 ± 1.5	-0.2 ± 0.9 [1]	0.829
VAT mass (g)	23.6 ± 108.8	54 ± 62.3 [1]	0.139
VAT volume (cm^3)	25.5 ± 117.8	61.2 ± 67.6 [1]	0.126
VAT area (cm^2)	4.9 ± 22.5	11.2 ± 12.9 [1]	0.126
GLP-1 (pM)	0.3 ± 0.9	-0.6 ± 0.9 [1]	0.012
GIP (pg/ml)	4.3 ± 40.1	-22.1 ± 40.1 [2]	0.077
Free testosterone (pmol/l)	0 ± 2.7	3.9 ± 4.2	0.022
Total testosterone (nmol/l)	0 ± 0.4	0.3 ± 0.7	0.274
Androstenedione (nmol/l)	0.4 ± 1.7	0 ± 1.4	0.525
DHEAS (µmol/l)	0 ± 0.8	0.4 ± 1.2	0.440
SHBG (nmol/l)	-6.6 ± 22.2	-8.5 ± 39.9 [1]	0.277
Number of periods in 3 months	-0.2 ± 0.7	-0.1 ± 0.9	0.808

BMI, body mass index; HOMA-B, homeostasis model assessment for beta-cell function; MBCI, modified beta-cell function index; QUICKI, quantitative insulin sensitivity check index; Glu, glucose; OGTT, oral glucose tolerance test; HOMA-IR, homeostasis model assessment of insulin resistance; IAI insulin action index; DXA Dual Energy X-ray Absorptiometer; VAT visceral adipose tissue; GLP-1 glucagon-like peptide-1; GIP glucose-dependent insulinotropic peptide; DHEAS dehydroepiandrosterone sulphate SHBG, sex hormone-binding globulin

For each parameter, missing data are presented in brackets []

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