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Metabolism Clinical and Experimental 58 (2009) 586 – 593
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Incretin hormone secretion in women with polycystic ovary syndrome: roles of obesity, insulin sensitivity, and treatment with metformin

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Received 2 June 2008; accepted 6 November 2008

Abstract

In normal subjects, the incretin hormones glucagon-like peptide–1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are responsible for 70% of the insulin response during a meal; but in diabetic subjects and other insulin-resistant conditions, the incretin effect is impaired. Polycystic ovary syndrome (PCOS) is associated with insulin resistance, and the pathophysiologic mechanisms behind PCOS resemble those of type 2 diabetes mellitus; therefore, women with PCOS may have alterations in the incretin hormone response. Metformin is widely used in the treatment of both type 2 diabetes mellitus and PCOS. Metformin may exert some of its effect on glucose metabolism by increasing GLP-1 biosynthesis and secretion and thereby increasing the incretin effect. The objective of the study was to measure incretin hormone secretion in women with PCOS and to evaluate the effect of metformin treatment. Cross-sectional comparison of 40 women with PCOS (19 lean and 21 obese) and 26 healthy control women (9 lean and 17 obese) and longitudinal evaluation of the effects of 8 months of metformin 1000 mg twice daily in women with PCOS were performed. Plasma concentrations of GIP and GLP-1 were determined frequently during a 75-g glucose tolerance test, and insulin sensitivity was evaluated by the euglycemic hyperinsulinemic clamp. The incretin hormone response did not differ between subjects with and without PCOS. Subgroup analysis showed lower GIP (area under the curve [AUC]) levels in obese women with PCOS compared with obese control women ($P < .05$) and compared with lean women with PCOS ($P < .05$). Metformin increased GIP (AUC) and GLP-1 (AUC) in lean women with PCOS ($P < .05$), and a similar trend was seen in the obese women ($P = .07$). The GIP secretion is attenuated in obese women with PCOS, whereas treatment with metformin increases the levels of both GIP and GLP-1 in women with PCOS.

1. Background

The incretin effect describes the phenomenon that oral ingestion of glucose elicits greater increases in insulin secretion than glucose administered via the intravenous route to reach the same plasma concentration [1-4]. This effect is primarily explained by the 2 incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide–1 (GLP-1). Several conditions involving insulin resistance and glucose intolerance have been associated with altered incretin effect [5-9]. These include type 2 diabetes mellitus, where the key observation is that the GLP-1 response to a mixed meal is blunted, whereas the GIP response is normal or slightly reduced [6]. An increased secretion of GIP, but a normal secretion of GLP-1, has been found in first-degree relatives of patients with type 2 diabetes mellitus [9], indicating that the defective incretin secretion is a secondary phenomenon. There has been some debate about the effect of obesity on the incretin effect [10-16]. In a recent study, Muscelli et al [17] found that obesity and glucose intolerance attenuate the GLP-1 response to an oral glucose tolerance test (OGTT). In HIV-infected male patients on highly active antiretroviral therapy, who are at high risk for developing insulin resistance, a larger as well as a blunted GLP-1 response to oral glucose has been found [7]. Finally, normal secretion and action of GLP-1 and GIP are found in subjects born small for gestational age, representing another insulin-resistant phenotype with a higher risk of developing type 2 diabetes mellitus [5].

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0026-0495/$ – see front matter © 2009 Published by Elsevier Inc.
doi:10.1016/j.metabol.2008.11.009

Polycystic ovary syndrome (PCOS) is a very common disease in young women at fertile age. The syndrome is characterized by anovulation, hyperandrogenemia/hyperandrogenism, and polycystic ovaries. It is a complex disease, and several pathophysiologic mechanisms seem to be involved. Insulin resistance and hyperinsulinemia play a central role in the pathogenesis [18-26], and women with PCOS are considered at increased risk of developing impaired glucose tolerance and type 2 diabetes mellitus [27-29]. Few previous studies have addressed the incretin effect in women with PCOS. In a recent study, Vrbikova et al [30] described higher levels of GIP in women with PCOS. The GLP-1 responses were identical in women with and without PCOS in the early phases of an OGTT, but lower levels were found in the late phases of the test [30].

Metformin belongs to the biguanide class of oral antidiabetic agents that improve glucose levels. It is widely used in the treatment of both type 2 diabetes mellitus and PCOS. The aim of the present study was to evaluate the GLP-1 and GIP responses to oral glucose in lean and obese women with PCOS and in weight-matched control women and to evaluate the effect of 8 months of metformin treatment on GIP and GLP-1 secretion in lean and obese women with PCOS.

2. Material and methods

Sixty-six women were included in the study after advertising in a local newspaper: 40 (21 obese and 19 lean) women with PCOS and 26 age-matched (17 obese and 9 lean) control women. Women with a body mass index (BMI) of at least 25 were classified as obese, and women with a BMI less than 25 were classified as lean. All control women had regular menstrual cycles (<35 days) and androgen levels within the reference range. Women with other known chronic diseases and women who had used oral contraceptives or other drugs known to alter glucose and insulin metabolism within the last 3 months were excluded from the study. The study was conducted according to the principles expressed in the Declaration of Helsinki and was approved by the local ethic authorities. All subjects gave written informed consent before entering the study. A menstrual history and history of general health were obtained. The PCOS diagnosis was based on the Rotterdam criteria [31]. Blood samples were taken for testosterone, and sex hormone–binding globulin (SHBG) and other endocrinopathies were excluded by relevant testing. Hirsutism was evaluated by the Ferriman-Gallwey [32] score, and women with a score of at least 8 were classified as having hirsutism. Polycystic ovary morphology was determined by transvaginal ultrasonography and categorized in accordance with the Rotterdam criteria [31].

3. Oral glucose tolerance test

After a 10-hour overnight fast, a standard OGTT (75 g) was performed. Blood samples were drawn from the antecubital vein at 0, 15, 30, 45, 60, 90, 120, and 180 minutes for measurement of plasma glucose concentrations, plasma insulin, total GIP, and total GLP-1.

3.1. Euglycemic hyperinsulinemic clamp

After an overnight fast of 10 to 12 hours, all women reported to our laboratory between 8:00 AM and 8:30 AM and were instructed to abstain from any strenuous physical activity for 3 days before the assessment. Catheters were inserted in the right and left antecubital vein and were used for blood sampling and infusion. The right arm was kept in a heating box to arterialize the venous blood. Insulin (Actrapid; Novo Nordisk, Bagsværd, Denmark) infusion was started with a rate of 100 mU m^{-2} min^{-1} followed by a stepwise decline in infusion rate every third minute by 20 mU/min until an infusion rate of 40 mU/min was reached after 9 minutes. During the remaining 120 minutes, a constant infusion rate of 40 mU/min was maintained. Plasma glucose levels were measured every 5 minutes and kept at 5 mmol/L by adjusting glucose (200 g/L) infusion rate. Lean body mass was evaluated by dual x-ray absorptiometry (QDR-2000, S/N 2256, Hologic, Waltham, MA) and was used for determination of glucose disposal rate (GDR).

4. Metformin treatment

All women with PCOS were offered metformin treatment with a 4-week stepwise dose increment to 1000 mg twice a day, which was continued for 7 months. The last dose of metformin was administered the night before the day of assessment. All women were asked to record the daily dose of metformin in a diary. In case of severe and persistent adverse effects such as stomachaches, nausea, or vomiting, the study subjects were allowed to reduce the dose of metformin until the adverse effects were tolerable. Control visits were performed every 2 months, where diaries of study drug compliance and adverse effects were evaluated. At the final visit, we performed a transvaginal ultrasound examination and evaluated the body weight and degree of hirsutism; and blood samples were drawn for determination of androgen levels. The euglycemic hyperinsulinemic clamp and the OGTT were also repeated after the 8-month intervention period.

5. Laboratory analysis

Plasma glucose was measured by Beckman glucose analyzer (Ramcon, Fullerton, CA). Testosterone and dihydrotestosterone (DHT) were measured by radioimmunoassay after ether extraction and subsequent celite chromatography. The intra- and interassay variations were 8.2% and 13.8%, respectively, for testosterone and 9.1% and 11.0%, respectively, for DHT. The detection limit for both analyses was 0.05 nmol/L. Plasma concentration of SHBG was analyzed by a double monoclonal immunofluorometric assay (Auto-DELFIA; Wallac Oy, Turku, Finland). Intra- and interassay

Table 1
Differences in baseline characteristics between women with and without PCOS analyzed by a 2-way ANOVA and an interaction analysis

	Age	BMI (kg/m^2)	Menstrual cycle (d) (median, range)	Total testosterone (nmol/L)	Free testosterone (nmol/L)	SHBG (nmol/L)	Fasting plasma glucose (mmol/L)	Fasting insulin (pmol/L)	2-h plasma glucose (mmol/L)
LC (9)	30 ± 4	22 ± 1	28 (28-33)	1.49 ± 0.3	0.015 ± 0.01	104 ± 33	5.4 ± 0.5	39 ± 21	5.0 ± 0.9
LP (19)	28 ± 5	23 ± 2	91 (28-365)	2.10 ± 0.8*	0.033 ± 0.02**	69 ± 29**	5.5 ± 0.4	44 ± 24	6.0 ± 1.2*
OC (17)	30 ± 6	34 ± 3	28 (28-33)	1.39 ± 0.4	0.025 ± 0.01††	55 ± 21†††	5.6 ± 0.3†	63 ± 21†	6.5 ± 1.2†
OP (21)	29 ± 4	32 ± 4	73 (28-183)	2.36 ± 0.8***	0.042 ± 0.02**	60 ± 37	5.9 ± 0.7†	69 ± 50	6.8 ± 1.2†
2-way ANOVA									
PCOS				<.0001	<.001	.06	.1	.5	<.05
Obesity				.6	.05	<.001	<.05	<.01	<.001
Interaction				.3	.9	.02	.4	1.0	.2

Mean ± SD, 2-way ANOVA, and Student *t* test. LC indicates lean controls; LP, lean PCOS; OC, obese controls; OP, obese PCOS.
*$P < .05$, **$P < .01$, and ***$P < .001$; vs BMI-matched controls.
$^{\dagger}P < .05$, $^{\dagger\dagger}P < .01$, and $^{\dagger\dagger\dagger}P < .001$; obese controls vs lean controls, and obese PCOS vs lean PCOS.

variations were 5.2% and 7.5%. Free testosterone was estimated from measurement of SHBG, total testosterone, and DHT by using the law of mass action including a calculation of testosterone binding to albumin [33]. Insulin was analyzed by 1235 AutoDELFIA automatic immunoassay system (Wallac Oy) with a detection limit of 3 pmol/L. The intra- and interassay coefficients of variance were 4.5% and 7%. The GIP and GLP-1 concentrations in plasma were measured after extraction of plasma with 70% ethanol (vol/vol, final concentration). For the GIP radioimmunoassay [34], we used the C-terminally directed antiserum R 65, which cross-reacts fully with human GIP but not with the so-called GIP 8000, whose chemical nature and relationship to GIP secretion are uncertain. The antiserum reacts equally with intact GIP and GIP 3-42, the primary metabolite. Human GIP and 125-I human GIP (70 MBq/nmol) were used for standards and tracer. The plasma concentrations of GLP-1 were measured [35] against standards of synthetic GLP-1 7-36amide using antiserum code no. 89390, which is specific for the amidated C-terminus of GLP-1 and therefore mainly reacts with GLP-1 of intestinal origin. The assay reacts equally with intact GLP-1 and with GLP-1 3-36amide, the primary metabolite. Because of the rapid and intravascular conversion of both GIP and GLP-1 to their primary metabolites, it is essential to determine both the intact hormone and the metabolite for estimation of the rate of secretion of these hormones. For all 3 assays, sensitivity was less than 1 pmol/L; intraassay coefficient of variation was less than 6% at 20 pmol/L; and recovery of standard, added to plasma before extraction, was about 100% when corrected for losses inherent in the plasma extraction procedure.

6. Calculations

The total and incremental areas under the curve (AUCs) for glucose, C-peptide, insulin, GIP, and GLP-1 were calculated for the time 0 to 180 minutes by use of the trapezoidal rule. Only the total AUC for the different variables is described. Peripheral insulin sensitivity was evaluated from the glucose infusion rate equal to GDR, determined during the steady-state period of the clamp after 90 to 120 minutes, and presented as milligrams per kilogram fat-free mass (FFM) per minute.

7. Statistical analysis

Results are presented as mean ± SD or SEM, except for data on menstrual cycle, which are presented as median

Table 2
Differences in metabolic parameters between women with and without PCOS analyzed by a 2-way ANOVA and an interaction analysis

	GDR (mg kg FFM^{-1} min^{-1})	Glucose $AUC_{0\text{-}180\ min}$ (mmol L^{-1} min^{-1})	Insulin $AUC_{0\text{-}180\ min}$ (nmol L^{-1} min^{-1})	C-peptide $AUC_{0\text{-}180\ min}$ (nmol L^{-1} min^{-1})	GIP $AUC_{0\text{-}180\ min}$ (pmol L^{-1} min^{-1})	GLP-1 $AUC_{0\text{-}180\ min}$ (pmol L^{-1} min^{-1})
LC (9)	13.3 ± 2.1	978 ± 125	26 ± 15	302 ± 59	5890 ± 2252	2212 ± 882
LP (19)	10.4 ± 3*	1193 ± 182	47 ± 26*	389 ± 126*	6266 ± 2337	2599 ± 1095
OC (17)	8.1 ± 2.8†††	1181 ± 162	50 ± 17††	407 ± 122	6638 ± 2667	2424 ± 1297
OP (21)	7.3 ± 2.8††	1267 ± 166	58 ± 35	414 ± 96	4975 ± 2078*†	2297 ± 779
2-way ANOVA						
PCOS	$P < .05$	<.001	<.05	.1	.3	.6
Obesity	$P < .0001$	<.01	<.05	<.05	.5	.7
Interaction	.1	.1	.4	.2	.07	.4

Mean ± SD, 2-way ANOVA, and Student *t* test.
*$P < .05$, **$P < .01$, and ***$P < .001$; vs BMI-matched controls.
$^{\dagger}P < .05$, $^{\dagger\dagger}P < .01$, and $^{\dagger\dagger\dagger}P < .001$; obese controls vs lean controls, and obese PCOS vs lean PCOS.

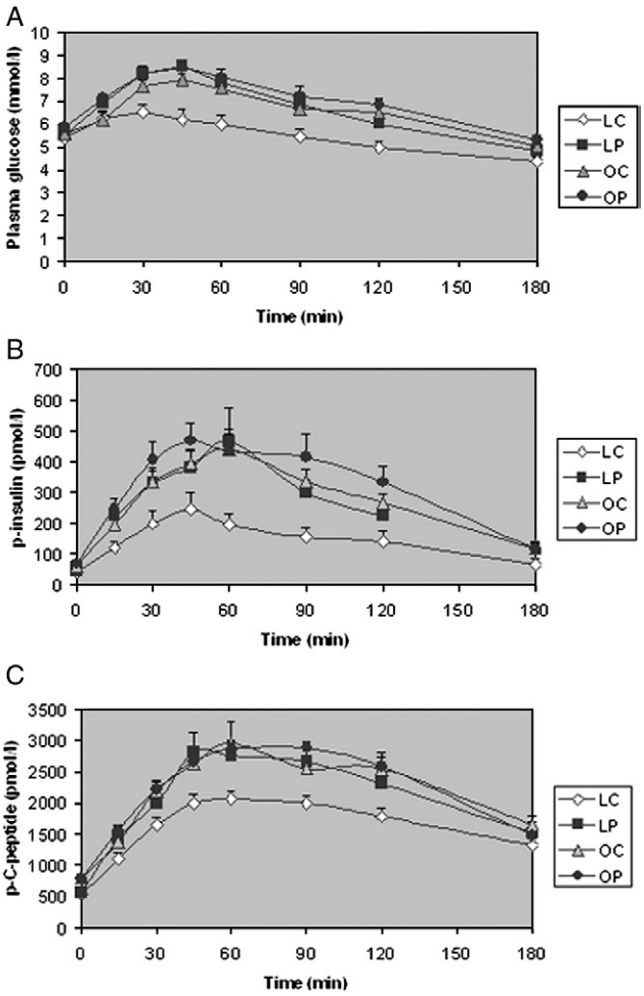

Fig. 1. Plasma glucose levels (A), insulin (B), and C-peptide (C) secretion during a 180-minute OGTT in lean and obese women with and without PCOS.

(range). A 2-way analysis of variance (ANOVA) was performed to determine the independent effects of PCOS and obesity and a possible interaction between PCOS and obesity on the different variables. A Student *t* test was performed for comparison of subgroups. Pre-and post-interventional data were compared by paired *t* test. Data on GIP and GLP-1 did not follow a Gaussian distribution and were therefore log-transformed and thereby approximated the normal distribution. Levels of significance were set at 0.05%.

8. Results

In the total study population, women with and without PCOS did not differ regarding age and BMI (Table 1). All control women had regular menstrual cycles. Nine (3 lean and 6 obese) women with PCOS had regular menstrual cycles, and the remaining had oligo- or amenorrhoea.

Polycystic ovary syndrome was associated with a significantly higher total ($P < .0001$) and free testosterone ($P < .001$), independent of obesity (Table 1). When stratified according to BMI, all 4 groups were similar regarding age; and BMI did not differ between the 2 lean and the 2 obese groups (Table 1). In the lean women, those with PCOS had significantly higher total ($P < .05$) and free ($P < .01$) plasma testosterone levels and lower plasma levels of SHBG ($P < .01$) compared with lean control women (Table 1). In the obese women, we also found significantly higher levels of both total ($P < .0001$) and free plasma testosterone levels ($P < .01$) in women with PCOS compared with control women (Table 1), whereas SHBG levels were similar.

8.1. Glucose and insulin metabolism

Polycystic ovary syndrome was associated with higher 2-hour plasma glucose levels during the OGTT ($P < .05$), whereas both fasting plasma glucose and insulin did not differ (Table 1). Polycystic ovary syndrome was associated with significantly lower GDR ($P < .05$) and higher AUC glucose ($P < .001$) and AUC insulin ($P < .05$) response during the OGTT (Table 2, Fig. 1A and B).

In the subgroup analysis, lean women with and without PCOS had identical fasting plasma glucose and insulin levels, but women with PCOS had higher 2-hour plasma glucose levels ($P < .05$), lower GDR values ($P < .05$), and higher insulin (AUC) and C-peptide (AUC) ($P < .05$) in response to the OGTT (Tables 1 and 2, Fig. 1B and C). In the obese subgroups, fasting plasma insulin, fasting plasma glucose, 2-hour plasma glucose, insulin (AUC), C-peptide (AUC), and GDR values did not differ between women with and without PCOS (Tables 1 and 2, Fig. 1B and C). Thus, PCOS is associated with insulin resistance and glucose

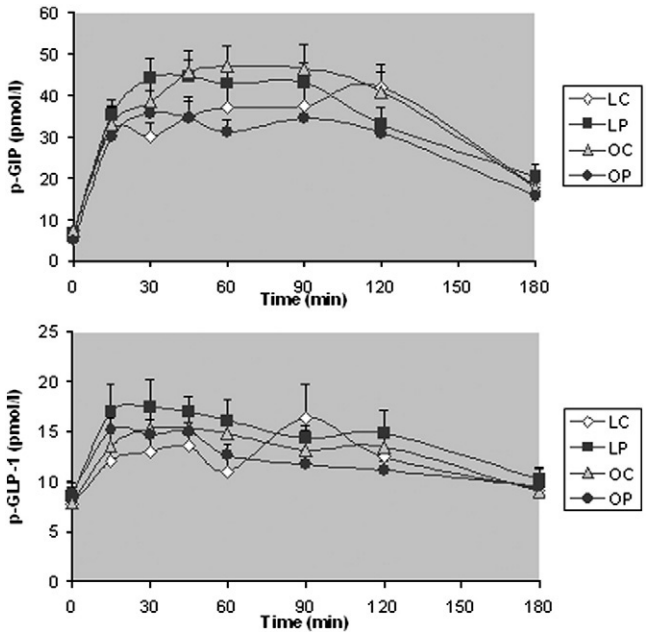

Fig. 2. Secretion of GIP and GLP-1 during a 180-minute OGTT in lean and obese women with and without PCOS.

Table 3
Baseline data for completers and noncompleters

	Completers (all PCOS, n = 22)	Noncompleters (all PCOS, n = 18)	P	Completers (LP, n = 10)	Noncompleters (LP, n = 9)	P	Completers (OP, n = 12)	Noncompleters (OP, n = 9)	P
GIP ($AUC_{0\text{-}180\ min}$, pmol L^{-1} min^{-1})	6328 ± 2480	4717 ± 2086	.06	6919 ± 927	5540 ± 407	.5	5653 ± 560	4073 ± 668	.08
GLP-1 ($AUC_{0\text{-}180\ min}$, pmol L^{-1} min^{-1})	2602 ± 870	2429 ± 1095	.6	2681 ± 336	2508 ± 396	.7	2444 ± 211	1868 ± 252	.09
Age (y)	28 ± 5	29 ± 3	.4	26 ± 2	30 ± 1	.1	29 ± 1	30 ± 1	.6
BMI (kg/m^2)	29 ± 6	26 ± 4.8	.2	24 ± 1	23 ± 1	.5	33 ± 1	32 ± 1	.7
Total testosterone (nmol/L)	2.3 ± 0.8	2.1 ± 0.9	.5	2.2 ± 0.3	2.0 ± 0.2	.7	2.5 ± 0.2	2.2 ± 0.3	.4
Free testosterone (nmol/L)	0.04 ± 0.02	0.03 ± 0.01	.1	0.04 ± 0.01	0.03 ± 0.01	.2	0.05 ± 0.01	0.03 ± 0.01	.05
Menstrual cycle (d), (median, range)	122 (28-365)	67 (28-183)	.3	128 (28-365)	91 (28-183)	.7*	122 (28-183)	61 (28-183)	.3*
Fasting plasma glucose (mmol/L)	5.7 ± 0.6	5.7 ± 0.3	.9	5.4 ± 0.1	5.6 ± 0.1	.08	5.9 ± 0.2	5.9 ± 0.2	1.0
Fasting plasma insulin (pmol/L)	51 ± 22	67 ± 61	.3	39 ± 7	50 ± 9	.3	58 ± 6	83 ± 24	.3
2-h plasma glucose (mmol/L)	6.5 ± 1.1	6.4 ± 1.4	.7	6.1 ± 0.4	5.9 ± 0.4	.8	6.6 ± 0.4	7.1 ± 0.4	.4
GDR (mg kg FFM^{-1} min^{-1})	8.3 ± 2.4[a]	9.4 ± 4.1[a]	.5	10.0 ± 0.5[a]	11.0 2.0[a]	.5	6.0 ± 0.6[a]	8.1 ± 1.3[a]	.3

[a] n = 15 (7 lean and 8 obese).
* Mann-Whitney test.

intolerance independently of obesity. This was confirmed in the comparison of lean women with and without PCOS. However, when obesity develops, the effect of PCOS on insulin resistance and glucose tolerance seems to be blunted.

8.2. Incretin hormone responses

In the total study population, the GLP-1 and GIP responses did not differ between women with PCOS and control subjects (Table 2, Fig. 2). In the subgroup analysis, both GIP (AUC) and GLP-1 (AUC) tended to be higher in lean women with PCOS compared with lean control women; but none of the comparisons reached significance. In the obese women with PCOS, GIP (AUC) was lower than in both weight-matched control women ($P < .05$) and in lean women with PCOS ($P < .05$) (Table 2, Fig. 2).

8.3. Metformin intervention

Twenty-two of the 40 women with PCOS completed the 8-month metformin treatment, and 18 did not. Of these 18 women, 7 either declined or stopped treatment because of adverse effects, 10 women became pregnant, and 1 moved to a different part of the country. Baseline data in dropouts and completers were comparable (Table 3). Only 15 (7 lean and 8 obese) of the 22 subjects completing the metformin treatment accepted repeated clamp examination at study termination. The baseline data from the 7 dropouts (3 lean and 4 obese) were not different from the completers in regard to any of the relevant metabolic parameters (data not shown).

Treatment with metformin increased both GIP (AUC) ($P < .05$) and GLP-1 (AUC) ($P < .05$), but there was no effect on either measures of insulin and glucose metabolism or

Table 4
Baseline and follow-up data on obese and lean women with PCOS

	All PCOS (n = 22)		Lean PCOS (n = 10)		Obese PCOS (n = 12)	
	Baseline	After metformin	Baseline	After metformin	Baseline	After metformin
Total testosterone (nmol/L)	2.3 ± 0.8	1.9 ± 0.8	2.2 ± 0.9	1.9 ± 0.9	2.5 ± 0.7	1.9 ± 0.7*
Free testosterone (nmol/L)	0.04 ± 0.02	0.05 ± 0.09	0.04 ± 0.02	0.03 ± 0.01	0.05 ± 0.02	0.07 ± 0.1
SHBG (nmol/L)	53 ± 28	46 ± 23	62 ± 30	59 ± 25	46 ± 26	35 ± 15
Fasting plasma glucose (mmol/L)	5.7 ± 0.6	5.6 ± 0.4	5.4 ± 0.3	5.5 ± 0.4	5.9 ± 0.7	5.6 ± 0.5
2-h plasma glucose (mmol/L)	6.5 ± 1.2	6.9 ± 1.2	6.1 ± 1.2	6.4 ± 1.4	6.9 ± 1.1	7.3 ± 0.9
Glucose $AUC_{0\text{-}180}$ (mmol L^{-1} min^{-1})	1239 ± 160	1310 ± 131*	1198 ± 172	1265 ± 165	1274 ± 149	1348 ± 85
Fasting insulin (pmol/L)	51 ± 22	53 ± 31	39 ± 21	39 ± 23	61 ± 18	65 ± 33
Insulin $AUC_{0\text{-}180\ min}$ (nmol L^{-1} min^{-1})	53 ± 26	51 ± 22	42 ± 20	38 ± 12	62 ± 28	61 ± 23
C-peptide $AUC_{0\text{-}180min}$ (nmol L^{-1} min^{-1})	410 ± 88	411 ± 100	388 ± 103	369 ± 89	427 ± 72	445 ± 98
GDR (mg kg FFM^{-1} min^{-1})	8.1 ± 2.7[a]	8.4 ± 2.3[a]	10.0 ± 1.9[b]	10.3 ± 1.4[b]	6.5 ± 2.2[c]	6.8 ± 1.5[c]
GIP $AUC_{0\text{-}180\ min}$ (pmol L^{-1} min^{-1})	6228 ± 2465	7954 ± 3009**	6919 ± 2933	9419 ± 3232*	5653 ± 1941	6733 ± 2272
GLP-1 $AUC_{0\text{-}180\ min}$ (pmol L^{-1} min^{-1})	2552 ± 881	3208 ± 760**	2681 ± 1061	3537 ± 572*	2444 ± 730	2933 ± 810

Mean and SD, paired *t* test.
[a] n = 15.
[b] n = 7.
[c] n = 8.
* $P < .05$.
** $P < .01$.

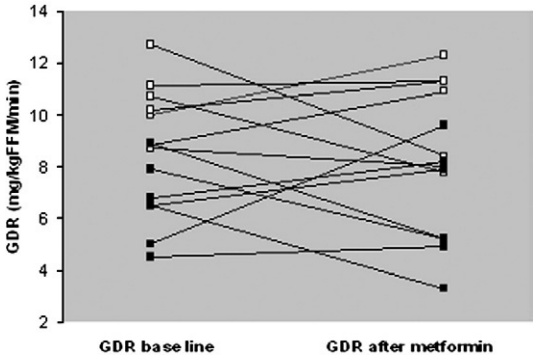

Fig. 3. Glucose disposal rate before and after metformin intervention in lean and obese women with PCOS. Open boxes, lean PCOS; closed boxes, obese PCOS.

androgen levels (Table 4, Figs. 3 and 4). In the weight-stratified groups, total testosterone levels decreased in obese women with PCOS ($P < .05$) (Table 4); but androgens were unchanged in the lean women with PCOS after metformin treatment. Free plasma testosterone levels and plasma SHBG levels were similar in both lean and obese women with PCOS before and after metformin (Table 4).

In neither lean nor obese women with PCOS did metformin have effects on the different measures of glucose and insulin metabolism; but in lean women with PCOS, both GIP (AUC) ($P < .05$) and GLP-1 (AUC) ($P < .05$) increased after treatment. In the obese group, we also found an increase of both GIP (AUC) and GLP-1 (AUC), which however only reached borderline significance ($P = .07$ for both GIP and GLP-1) (Table 4, Figs. 3 and 4).

9. Discussion

We have investigated insulin sensitivity and the secretion of the 2 incretin hormones GIP and GLP-1 during an OGTT in lean and obese women with and without PCOS and in lean and obese women with PCOS before and after 8 months of metformin treatment. Women with PCOS displayed significantly higher 2-hour plasma glucose levels, higher insulin levels, and lower insulin sensitivity, independent of obesity (Table 2), which are in accordance with previous results [19,36]. The GLP-1 and GIP response during the OGTT did not differ between women with and without PCOS. Furthermore, the presence of obesity did not seem to have any effect on incretin hormone responses. In a recent study, Muscelli et al [17] showed an independent effect of BMI on the incretin effect. They studied a population of both men and women, all obese and older than our study population. They also used different measures of the incretin function, which include the action of the incretin hormone on insulin secretion. All these factors may explain the discrepancy between their results and our results.

In our subgroup analysis, we found that GIP and GLP-1 responses were similar in lean women with and without PCOS; but there was a trend toward a slightly increased incretin hormone response in lean women with PCOS

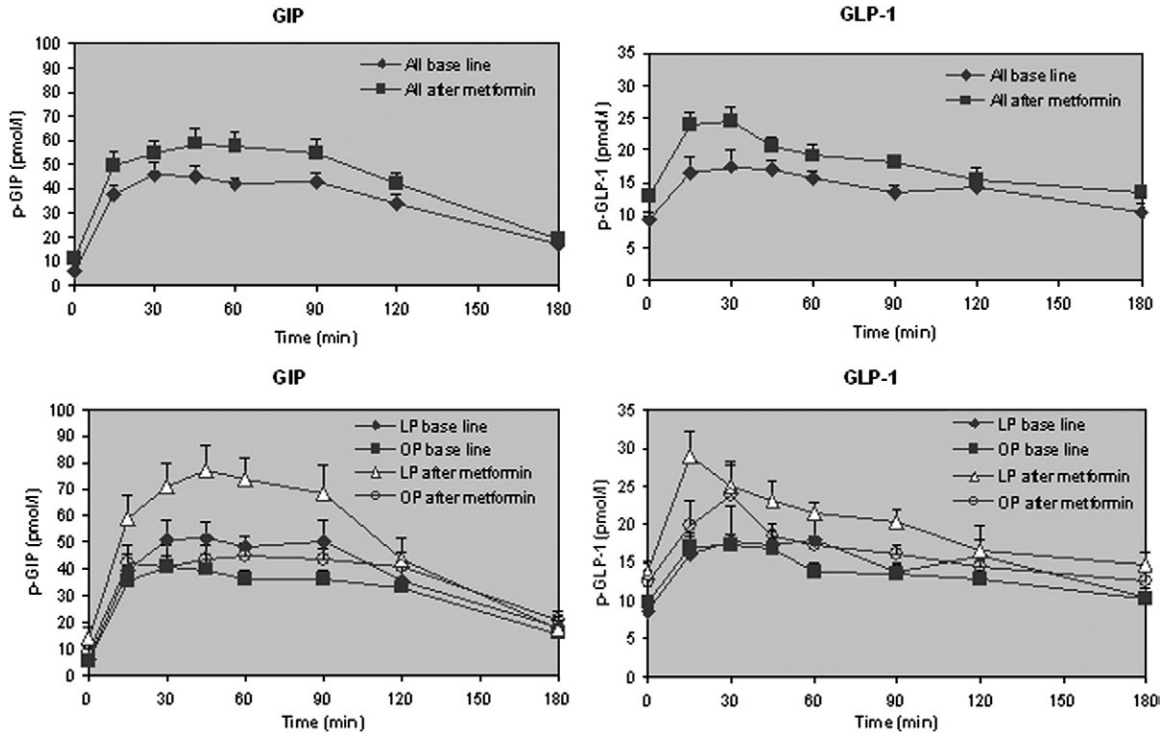

Fig. 4. . The time course of GIP and GLP-1 secretion during a 180-minute OGTT before and after 8 months of metformin intervention in women with PCOS.

compared with lean control women. This is partly in accordance with the findings of Vrbikova et al [30]. They found significantly higher levels of GIP, but significantly lower levels of GLP-1, in the late phases of the OGTT in PCOS compared with controls. This incongruence may be explained by the use of different assays and different study populations. In our study, lean women with PCOS had significantly lower insulin sensitivity than control women, whereas Vrbikova et al found similar insulin sensitivity in lean women with and without PCOS.

A further increased incretin hormone response in obese control women, who were more insulin resistant than lean women with PCOS, was found; and although we found a marked decrease in incretin secretion in obese women with PCOS, it had no obvious effect on their insulin secretion. This is in accordance with previous findings of Meier et al [37]. They investigated the decelerating effect of GLP-1 on gastric emptying by using a panel of prokinetic drugs and found that insulin secretion was similar between the experiments with administration of placebo and of GLP-1, alone or in combination with a prokinetic erythromycin [37].

Metformin is widely used in women with PCOS, and its proposed effect on ovulation and androgen levels is assumed to be due to the effect on insulin sensitivity. In the present study, metformin had no effect on insulin sensitivity. We did, however, show a lowering effect on total plasma testosterone levels in both lean and obese subjects; but it was only significant in the obese group. These results are in accordance with what has been found in a previous study by Lord et al [38]. They found no effect of 12 weeks of metformin intervention on insulin sensitivity and androgen levels in women with PCOS, whereas previous investigators have shown a beneficial effect of metformin on both insulin sensitivity and androgen levels [39-41]. Different diagnostic criteria for PCOS (and thereby different study populations), different baseline degrees of glucose tolerance and insulin sensitivity, and different measures of insulin sensitivity may explain these contrasting results. Furthermore, metformin primarily exerts its effect on liver insulin sensitivity, which may not be detected in a euglycemic clamp assessing primarily peripheral insulin sensitivity. Ten women became pregnant during the intervention period; and although their baseline data on insulin sensitivity did not differ from the 22 women who completed the study, we cannot exclude that these women had a higher response rate to metformin than the completers. Therefore, our results may be slightly hampered by selection bias.

Despite the lack of improvement in insulin sensitivity, we found a significant increase in both GIP and GLP-1 in lean women and a tendency to an increase of GLP-1 and GIP responses in obese women with PCOS. It has been suggested that metformin increases plasma concentrations of GLP-1 both by increasing GLP-1 biosynthesis and secretion [42] and also by inhibiting DDP-IV, thereby inhibiting GLP-1 degradation [43-46]. We also found an increase in GIP after metformin treatment, which has not been found previously. Surprisingly, the increase in incretin secretion did not seem to be reflected in postprandial glucose tolerance.

We conclude that there is no independent effect of PCOS on the incretin response. However, incretin secretion is attenuated in obese women with PCOS compared with lean women with PCOS and obese control women. Furthermore, metformin treatment increases levels of both GIP and GLP-1 in women with PCOS. There are some limitations to this study, as we have only focused on incretin secretion and not the incretin effect; and it would be relevant to also investigate the incretin effect in future studies.

Acknowledgment

We would like to thank The Danish Diabetes Association for economic support, and Susanne Reimar and Lene Brus Albæk for technical assistance.

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