CLINICAL STUDY

Incretin levels in polycystic ovary syndrome

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Abstract

Objective: Polycystic ovary syndrome (PCOS) has been linked to a high risk of type 2 diabetes mellitus. Disturbances in the secretion of the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) have been observed in states with impaired glucose regulation. This paper considers the secretion of GIP and GLP-1 after oral glucose load in a group of lean, glucose-tolerant PCOS women in comparison with age- and body mass index (BMI)-matched healthy women.

Design: Case control.

Methods: PCOS ($n=21$, 25.8 ± 4.1 years, BMI 21.6 ± 1.7 kg/m²) and control healthy women (CT, $n=13$, 28.5 ± 7.2 years, BMI 20.3 ± 2.5 kg/m²) underwent oral glucose tolerance test (OGTT) with blood sampling for glucose, insulin, C-peptide, total GIP, and active GLP-1. Insulin sensitivity was determined both at fasting and during the test.

Statistics: Repeated measures ANOVA.

Results: Glucose levels and insulin sensitivity did not differ between PCOS and CT. PCOS had significantly higher levels of C-peptide ($P<0.05$) and tended to have higher insulin levels. The levels of total GIP were significantly higher in PCOS than in CT ($P<0.001$). Active GLP-1 levels exhibited a significantly different time-dependent pattern in PCOS ($P<0.002$ for PCOS versus time interaction). GLP-1 concentrations were similar in PCOS and CT in the early phase of OGTT and then reached significantly lower levels in PCOS than in CT at 180 min ($P<0.05$).

Conclusions: Increased total GIP and lower late phase active GLP-1 concentrations during OGTT characterize PCOS women with higher C-peptide secretion in comparison with healthy controls, and may be the early markers of a pre-diabetic state.

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Introduction

Incretins are peptide hormones, secreted from the gut, responsible for the augmentation of insulin secretion after oral glucose intake (1, 2). Glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), formerly known as gastric inhibitory polypeptide (GIP), are the most important of these.

An altered secretion pattern and/or insulinotropic activity of incretins have been described in diabetes and in other conditions connected with impaired glucose regulation. Impaired secretion of GLP-1, preserved insulinotropic GLP-1 activity, and, by contrast, loss of the insulinotropic action of GIP with normal or only moderately impaired GIP secretion have been seen in patients with type 2 diabetes mellitus (T2DM) (3). In first-degree relatives of T2DM patients, GLP-1 secretion was unchanged and GIP responses after meals or OGTT were on average higher than in healthy control subjects (4). Furthermore, increased GIP levels have been observed in

obese subjects (5) and both GIP and insulin secretion decreased after successful gastric bypass (6). Similarly, increased GIP levels have been described in elderly subjects (7). The cited studies reflect the current marked interest in the evaluation of the role of incretin hormones in different metabolic states.

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders and affects 5–10% of women in their fertile years (8, 9). Women with PCOS have an increased prevalence of T2DM (10–12) and some studies suggest that patients with PCOS should undergo early screening for T2DM (13). With the exception of a small study demonstrating no differences in GIP and GLP-1 levels between PCOS and healthy subjects (14), to the best of the authors' knowledge, no study to date has evaluated the relationship of incretin secretion to β -cell function in PCOS. We therefore investigated the secretion of GIP and GLP-1 after oral glucose load in a group of lean glucose-tolerant PCOS women in comparison with healthy women matched for

body mass index (BMI) and age, with the aim of characterizing incretin release in a disorder known likely to be a pre-diabetic state.

Subjects and methods

Subjects

Of 34 lean PCOS women diagnosed according to the ESHRE consensus (15) at the Institute of Endocrinology between October 2005 and March 2007, 21 fulfilled the inclusion criteria of the study (a negative family history of T2DM, normal glucose tolerance, BMI <25 kg/m², and the exclusion of any other serious illness). The subjects had been free of any medication influencing glucose metabolism, and of hormonal contraception, for at least 3 months. In all of the women, oligoamenorrhea was present in combination with either an elevation of testosterone and the index of free testosterone above the upper limit of the normal range (e.g., 0.40–2.65 nmol/l for testosterone, free testosterone index <6) and with PCO morphology on transvaginal ultrasound (n=17), or with the PCO and normal androgen levels but with significant hirsutism (n=2), or with both elevation of the androgens and clinically significant hirsutism and normal/absent (as one subject refused to undergo ultrasound examination) ovarian ultrasound morphology (n=2).

Thirteen healthy women with normal glucose tolerance, recruited via advertisements, served as the control population (CT, Table 1). They were free of any medication affecting glucose metabolism, had negative family history of T2DM, and were also free of any family history of hyperandrogenic symptoms and infertility. CT had no symptoms of hyperandrogenism, had a regular menstrual cycle (21–35 days), and had androgen levels within the reference range. Pelvic ultrasonography was not performed in CT.

Tests and assays

After signing written informed consent approved by the local Ethical Committee, weight (to the nearest 0.1 kg) and height (to the nearest cm) were measured. Basal fasting blood samples for lipid profile and hormonal parameters were taken from the cubital vein between the first and fifth day of the spontaneous menstrual cycle or, if amenorrhea was present, at any day. Samples were centrifuged and serum was stored at –20 °C until analysis. OGTT was performed after overnight fasting by sampling blood before the 75 g glucose oral load and after 30, 60, 90, 120, 150, and 180 min. Samples for blood glucose, insulin, and C-peptide concentration measurements were centrifuged and serum frozen at –20 °C until analysis. GIP and GLP-1 were determined from samples at 0, 30, 60, 120, and 180 min. Blood was collected into pre-chilled tubes with EDTA. Immediately after sampling, 10 µl/ml dipeptidyl peptidase-IV (DPP-IV) inhibitor (Linco, St Charles, MO, USA) and 50 µl antilysin (Spofa, Prague, Czech Republic) were added, and the tubes were centrifuged at 2000 g for 10 min; plasma samples were immediately frozen at –70 °C until analysis.

Total cholesterol, high density lipoprotein (HDL)-cholesterol, and triglycerides were assessed by photometry (Ecoline 25, Merck Vitalab Eclipse). Plasma glucose was determined by the glucose oxidase method (Beckmann, Fullerton, CA, USA), with intra- and inter-assay coefficients of variation (CVs) of 1.8 and 2.6% respectively. C-peptide and insulin were assayed by IRMA (Immunotech, Prague, Czech Republic), with intra- and inter-assay CVs of 4.1 and 5.1% for C-peptide and 4.6 and 5.3% for insulin respectively. Testosterone (T) was determined by RIA with the use of our own antiserum (anti-testosterone-3-carboxymethyloxime: BSA), with intra- and inter-assay CVs of 10.2 and 10% respectively. Sex hormone-binding globulin (SHBG) was determined by IRMA (Orion, Espoo, Finland) with intra- and inter-assay CVs of 6.1 and 7.9% respectively. Active GLP-1 was

Table 1 Anthropometric and biochemical data and insulin sensitivity in women with polycystic ovary syndrome (PCOS) and controls (CT).

Variable	PCOS (n=21)		CT (n=13)		P value
	Mean	S.D.	Mean	S.D.	
Age (years)	25.81	3.98	28.46	6.90	NS
Body mass index (kg/m ²)	21.57	1.68	20.27	2.37	NS
Waist-to-hip ratio	0.75	0.05	0.75	0.04	NS
Total cholesterol (mmol/l)	4.02	0.74	4.21	0.41	NS
HDL-cholesterol (mmol/l)	1.74	0.39	1.64	0.37	NS
Triglycerides (mmol/l)	0.77	0.37	0.66	0.24	NS
Testosterone (nmol/l)	3.03	0.79	1.68	0.64	0.00009
SHBG (nmol/l)	52.05	15.50	60.83	26.78	NS
Free testosterone index	6.57	3.01	3.19	1.49	0.002
Basal hepatic insulin extraction (molar ratio)	0.71	0.50	0.67	0.58	NS
Hepatic insulin extraction during OGTT (%)	68.1	8.7	67.0	9.1	NS
Fasting insulin sensitivity (QUICKI)	0.39	0.04	0.39	0.03	NS
Dynamic insulin sensitivity (OGIS, ml/min per m ²)	536	69	556	53	NS

QUICKI, quantitative insulin sensitivity index; OGIS, oral glucose insulin sensitivity; SHBG, sex hormone-binding globulin.

determined by ELISA (Linco Research) with no cross reactivity with glucagon, GLP-2 or GLP-1 (9–36 amide). Intra- and inter-assay CVs were in the range of 6.7–7.8 and 1.8–6.1% respectively. Total GIP was determined by ELISA (Linco Research) with no cross-reactivity with oxyntomodulin, glucagon, GLP-1, or GLP-2. Intra- and inter-assay CVs were in the range of 7–9 and 1–13% respectively.

Calculations and statistics

Insulin sensitivity was assessed in fasting conditions with the quantitative insulin sensitivity index QUICKI (16), and in dynamic conditions with the oral glucose insulin sensitivity index (OGIS), which describes glucose clearance during the postprandial phase of the OGTT (17). These indices have been validated against the euglycemic hyperinsulinemic clamp and are widely used (18). Hepatic insulin extraction was computed in the basal state as the molar ratio of C-peptide to insulin levels, and during the OGTT according to a model derived method (19).

Comparisons were made using repeated measures ANOVA, with status (PCOS or CT) as the inter-subject factor and subject factor and time as the intra-subject factor and factor interaction, followed by least significant difference multiple comparisons. For multiple comparisons, a probability level of $P < 0.05$ was considered statistically significant. Spearman's correlations between C-peptide or insulin and GIP or GLP-1 levels at different time points of the OGTT were computed. Due to the skewed data distribution and heteroscedasticity in both the dependent variables and the residuals, the data were treated by a power transformation to approximate a Gaussian data distribution and constant variance. The outliers were detected using residual diagnostic plots. Statgraphics v. 5.1 statistical software (Rockville, MA, USA) was used for the computations.

Results

The clinical, biochemical, and hormonal parameters of the subjects are given in Table 1. Age, BMI, waist-to-hip ratio (WHR), total cholesterol, HDL-cholesterol, and triglyceride levels did not differ between PCOS and CT. Insulin sensitivity and hepatic insulin extraction, both at fasting and during OGTT, were similar in PCOS and CT. As expected, PCOS had significantly higher testosterone levels ($P < 0.00009$) and free testosterone index ($P < 0.002$) than CT, even when SHBG levels were not different between the groups.

OGTT time courses are shown in Fig. 1a and b. Blood glucose was similar in PCOS and CT ($P < 0.69$). Women affected with PCOS exhibited higher levels of C-peptide than CT ($P < 0.05$) and tended to have higher levels of insulin ($P < 0.12$; Fig. 1a). The concentrations of total GIP were significantly higher in PCOS than in CT

($P < 0.001$). Active GLP-1 levels exhibited a significantly different time-dependent pattern in PCOS than in CT ($P < 0.002$ for PCOS versus time interaction). GLP-1 concentrations were similar in PCOS and CT in the early phase of OGTT until 60 min and reached significantly lower levels in PCOS than in CT ($P < 0.05$) at 180 min (Fig. 1b).

C-peptide levels correlated significantly with total GIP levels at 0 ($r = 0.40$; $P < 0.04$), 60 ($r = 0.46$; $P < 0.01$), and 180 min ($r = 0.60$; $P < 0.002$). No significant correlation was observed between active GLP-1 and C-peptide. Neither total GIP nor active GLP-1 correlated with insulin.

Discussion

Incretin hormones, GIP and GLP-1, are responsible for the augmentation of insulin secretion after oral glucose load (1, 2). To the best of the authors' knowledge, there is only one previous study of incretin hormones in a small group of lean PCOS, which found no difference in GIP or GLP-1 between PCOS and controls, even when the PCOS women had higher insulin and C-peptide levels (14). By contrast, in the study presented here, women with PCOS exhibited increased total GIP and increased C-peptide concentrations during OGTT in comparison with healthy control subjects. On the other hand, active GLP-1 levels exhibited a significantly different time course, being similar in PCOS and controls in the early phase, but lower in PCOS in the late phase of OGTT. The observed discrepancies between the present results and those of the cited investigation (14) could be due to either the limited sample size of the previous study or the methodological differences in the incretin hormone assays. Our results do on the other hand accord with other authors, describing increased GIP (20) and decreased active GLP-1 levels in patients with overt T2DM (21). Similarly, increased total and intact GIP levels accompanied by increased C-peptide levels have been shown in subjects with impaired glucose tolerance (22). Increased concentrations of total GIP together with increased C-peptide secretion have also been found in healthy first-degree relatives of patients with T2DM during day-long sampling profiles (4). All these findings, together with those of the present study in PCOS, show that incretins, and especially GIP, seem to be altered both in overt diabetes and in those metabolic states considered as pre-diabetic.

The studied cohort of women affected with PCOS exhibited increased β -cell function, as they had significantly increased concentrations of C-peptide in comparison with matching healthy subjects. Similar to C-peptide, insulin levels also tended to be higher in PCOS, but did not reach significance, probably due to the greater dispersion of insulin levels. Liver degradation of the hormone should not be responsible for the discrepancy between insulin and C-peptide,

Figure 1 (a) Levels of glucose, C-peptide, and insulin after oral glucose load in women with polycystic ovary syndrome (closed circles, solid line) in comparison with age- and BMI-matched healthy women (open circles, hatched line). (b) Levels of total glucose-dependent insulinotropic polypeptide (GLP) and active glucagon-like peptide 1 (GLP-1) after oral glucose load in women with polycystic ovary syndrome (closed circles, solid line) in comparison with age- and BMI-matched healthy women (open circles, hatched line).

because there was no difference in hepatic insulin extraction, both at fasting and during OGTT, in the PCOS and controls. The increased β -cell response is not due to obesity, as the patients and controls were lean with similar BMI and WHR. We have chosen lean PCOS for this study specifically to avoid the possible confounding effect of obesity on β -cell release.

β -Cell compensation for the given degree of insulin resistance (23) should not be responsible for the observed differences, as both groups had the same degree of insulin sensitivity measured in both a fasting state and during dynamic conditions. Similar insulin sensitivity is consistent with other reports employing the euglycemic clamp (24, 25). Other authors have also described increased C-peptide levels during OGTT, which were not accompanied by decreased insulin sensitivity as measured by euglycemic clamp in PCOS women (26). Increased β -cell activity has been described in other populations with a high risk of T2DM. Early insulin responses in OGTT, meal test, and IVGTT were exaggerated in the Pima Indians, for example, and could not be explained by insulin resistance (27). It is possible to speculate that increased β -cell activity is an early defect that is present even before subtle defects in insulin sensitivity can be discerned.

We have shown that total GIP secretion was an important factor contributing to increased C-peptide levels, which significantly correlated with GIP levels, but not with active GLP-1, at different time points of OGTT. The role of GLP-1 must not, however, be neglected, because although GIP levels are up to ten times higher than GLP-1 levels, both incretins act in an additive manner, with GLP-1 being predominant at higher glucose concentrations (28). The reasons for the lack of correlation between GLP-1 and insulin or C-peptide levels are not clear. Decreased GLP-1 secretion has been found in patients with overt T2DM, where, however, GLP-1 had preserved insulinotropic activity (3). In the present study, GLP-1 exhibited a significantly different time pattern in PCOS than in CT. GLP-1 concentrations were similar in PCOS and CT in the early phase of OGTT until 60 min and reached significantly lower levels in PCOS than in CT at 180 min. In our opinion, this was an interesting observation, although we were not able to provide a clear explanation of this phenomenon. The regulation of GLP-1 secretion is still not fully clarified. Rate of gastric emptying, leptin, and glucagon have been shown to influence GLP-1 secretion (1). In particular, leptin stimulates GLP-1 secretion, and this effect was attenuated in subjects with leptin resistance (29), while glucagon correlated negatively with GLP-1 during OGTT in patients with T2DM. On the other hand, no relationships between the parameters of glucose control, insulin sensitivity, and secretion with GLP-1 levels have been found (30).

One of the hypotheses explaining increased GIP levels in PCOS would be primary overactivity of the

enteroinsular axis. Increased GIP secretion could exaggerate the stimulation of β -cells to produce more insulin. The inappropriately increased insulin secretion should lead to lower blood glucose levels if insulin sensitivity is preserved. Indeed, lower levels of glycated hemoglobin (31) and a lower perception of hypoglycemic symptoms (32) have been observed in women affected with PCOS. We cannot conclude from our study whether the increased GIP levels in PCOS are associated with the altered insulinotropic effect of GIP; this remains to be evaluated by the administration of GIP during hyperglycemic clamp and by measurement of the incretin effect. Alternatively, GIP levels could be increased as a compensatory mechanism of decreased GIP activity (3). The decreased GIP activity observed in T2DM was originally supposed to result from the loss-of-function mutations of the GIP receptor, but no association of causative polymorphism or mutations in GIP receptor and T2DM has been identified (33, 34). Decreased GIP activity and concomitantly increased plasma GIP levels might also result from the decreased/absent expression of GIP receptors on the β -cells. In humans, however, the defective GIP action is restricted to the late phase of insulin secretion with preserved early phase GIP activity. Increased internalization or enhanced desensitization of GIP receptors has, therefore, been suspected (3). The decreased insulinotropic effect of GIP has been demonstrated not only in diabetic subjects, but also in their first-degree relatives with normal glucose tolerance (35) and thus, the GIP defect has been supposed to be a primary, genetically determined defect in T2DM. When subjects with different types of diabetes were examined, however, decreased responses to GIP in a similar range as in T2DM were also found in subjects with MODY or with secondary diabetes (36). The defective GIP activity could therefore result from the diabetic metabolic state (37).

In conclusion, increased total GIP and lower late phase active GLP-1 concentrations during OGTT were observed in lean PCOS women compared with BMI- and age-matched control subjects.

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