An impaired glucagon-like peptide-1 response is associated with prediabetes in polycystic ovary syndrome with obesity

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Abstract
Objective: Impaired glucose homeostasis in polycystic ovary syndrome (PCOS) is associated with obesity, age, and disease phenotype. This study aimed to investigate the glucagon-like peptide-1 (GLP-1) response in patients with obesity and PCOS with normal glucose tolerance (NGT) or prediabetes.

Methods: Twenty-six women with obesity and PCOS were included. Thirteen women had NGT and 13 had prediabetes. Serum glucose, insulin, and GLP-1 levels were measured during an oral glucose tolerance test. Beta-cell function and insulin resistance were determined.

Results: Women with prediabetes had significantly lower GLP-1 levels than did those with NGT after a glucose load. GLP-1 levels <3.02 pM at 120 minutes were associated with prediabetes. Women with prediabetes had a lower oral glucose insulin sensitivity (OGIS) index and greater amount of visceral adipose tissue than did those with NGT. Plasma GLP-1 levels at 120 minutes were correlated with visceral adiposity and the OGIS index. A change in GLP-1 levels was correlated with a family history of type 2 diabetes.
**Conclusion:** The GLP-1 response is lower in patients with obesity, PCOS, and prediabetes than in those with obesity, PCOS, and NGT. Further investigation of the GLP-1 response as a potential separate risk factor for prediabetes in PCOS is required.

**Keywords**
Prediabetes, glucagon-like peptide-1 (GLP-1) response, obesity, polycystic ovary syndrome, normal glucose tolerance, insulin

Date received: 21 January 2019; accepted: 2 July 2019

**Introduction**

Impaired glucose homeostasis in polycystic ovary syndrome (PCOS) is closely associated with obesity, age, and disease phenotype. A body mass index (BMI) >30 kg/m², age older than 30 years, and disease phenotype A are the strongest risk factors associated with an adverse metabolic profile in the PCOS population. Conventionally, BMI is considered as the only modifiable risk factor for PCOS. Therefore, first-line treatment strategies in PCOS with obesity and high metabolic risk have focused on weight management. Despite established weight reduction strategies, the conversion rate from normal glucose tolerance (NGT) to prediabetes in obese women with PCOS remains two to three times higher compared with the expected conversion rate of 1% to 5% per year in the general obese population. Potential new modifiable risk factors should be addressed in this metabolically high-risk population.

Recently, mounting evidence has indicated that the incretin hormone glucagon-like peptide-1 (GLP-1) has an important role for maintaining normal glucose regulation. Loss of incretin effect in prediabetes, type 2 diabetes (T2D), and obesity has been shown in several clinical trials and incretin therapy is well established in these populations.

Although incretin-based therapy appears to be a reasonable therapeutic option in the subset of women with PCOS, data of the incretin effect in PCOS are limited and inconclusive. PCOS is related to either decreased or increased fasting and with lower postprandial GLP-1 levels. Studies on BMI as an independent risk factor for impairment of GLP-1 in PCOS have shown varied results. Some studies showed no difference in the GLP-1 response between lean and obese PCOS, whereas others reported lower GLP-1 levels in women with obesity. This study aimed to investigate the GLP-1 response in patients with obesity and PCOS with NGT or prediabetes.

**Subjects and methods**

**Subjects**
A case–control study was performed, including Caucasian women with obesity, who were recruited between January and March 2017 from the subspecialized outpatient clinics that are dedicated to patients with the cardiometabolic PCOS phenotype. All included women were treatment-naïve with phenotype A PCOS (based on the Rotterdam criteria consensus, which is characterized by concomitant presence of hyperandrogenemia at either the
biochemical or clinical level, menses abnormalities, and polycystic ovary morphology. Patients were included in the study if they met the inclusion criteria of a BMI >30 kg/m², aged 18 years to menopause, and without known T2D, and they were willing to participate in the study.

**Hormonal measurements**

Prediabetes was defined as having impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or both. The cut-off values were determined in a 75-g oral glucose tolerance test (OGTT) according to the World Health Organization 2006 criteria as follows: NGT (fasting glucose <6.1 mmol/L and 2-hour glucose <7.8 mmol/L), IFG (fasting glucose ≥6.1 mmol/L and <7.0 mmol/L, and 2-hour glucose <7.8 mmol/L), and IGT (fasting glucose <6.1 mmol/L, and 2-hour glucose ≥7.8 mmol/L and <11.1 mmol/L). The patients were matched for BMI and age (Table 1). Serum glucose, insulin, C-peptide, total GLP-1, and total glucose-dependent insulinotropic polypeptide (GIP) levels and the androgen profile were sampled during the OGTT. Glucose, C-peptide, and insulin levels were measured at 30-minute intervals (at 0, 30, 60, 90, and 120 minutes). Measurements of GLP-1 levels were performed at 0 and 120 minutes. The androgen and lipid profiles were measured at the start of the OGTT test. Glucose, insulin, C-peptide, and sex hormone levels were analyzed by routinely used automated methods. Total GLP-1 and GIP levels were measured in plasma using the commercially available kits Total GLP-1 ELISA (7-36 and 9-36) (catalog no. 43-GPTHU-E01; ALPCO, Salem, NH, USA) and Human GIP (Total) ELISA (EZHGIP-54K; EMD Millipore, St Louis, MO, USA), respectively. The homeostatic model assessment for beta-cell function-insulin secretion function index (HOMA-B), modified beta-cell function index (MBCI), and quantitative insulin sensitivity check index (QUICKI) were calculated. For insulin resistance (IR), homeostasis model assessment of insulin resistance (HOMA-IR) calculation and the insulin action index (IAI) were applied. For insulin sensitivity from the OGTT, the oral glucose insulin sensitivity (OGIS) index was used.

The formulas for calculating the above-mentioned parameters are as follows:

- \( \text{HOMA-B} = 20 \times \frac{I_0}{G_0 - 3.5} \)
- \( \text{MBCI} = \frac{I_0 \times G_0}{(G_{120} + G_{60} - 7)} \)
- \( \text{QUICKI} = 1 / \log (I_0 + \log(G_0)) \)
- \( \text{HOMA-IR} = I_0 \times \frac{G_0}{22.5} \)
- \( \text{IAI} = 1 / (I_0 \times G_0) \)

where \( I_0 \) denotes fasting plasma insulin, \( G_0 \) is fasting plasma glucose, \( G_{60} \) is plasma glucose levels at 60 minutes after a glucose load, and \( G_{120} \) is plasma glucose levels at 120 minutes after a glucose load in the OGTT test.

The OGIS index was calculated using the internet calculator available at: http://webmet.pd.cnr.it/OGIS/index.php. All patients underwent measurement of whole-body composition by a hologic dual energy X-ray absorptiometer (DXA). The study was approved by the 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 in the study. The study is registered at www.ClinicalTrials.gov as NCT03325569.

**Statistical analysis**

Calculation of the sample size was determined on the basis of differences in GLP-1 levels using Power and Sample Size Calculation version 3.0.43 (available at http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize). Data from comparable previous studies reported a 25% difference in GLP-1 levels between subjects with NGT and subjects with IGT. To detect a statistically significant difference in GLP-1 levels...
Table 1. Comparison of clinical characteristics of patients with NGT or IGT/IFG.

| Characteristic                      | NGT (n = 13) mean ± SD | IGT/IFG (n = 13) mean ± SD | p     |
|------------------------------------|------------------------|-----------------------------|-------|
| Age (years)                        | 37.1 ± 7.3             | 37.4 ± 6.7                  | 0.920 |
| Weight (kg)                        | 103.3 ± 14.2           | 99.2 ± 9.2                  | 0.650 |
| BMI (kg/m²)                        | 37.8 ± 6.9             | 36.2 ± 3.8                  | 0.579 |
| Waist circumference (cm)           | 101.5 ± 12.7           | 107.5 ± 10.7                | 0.223 |
| Glu at 0 minutes, OGTT (mmol/L)    | 5.3 ± 0.4              | 6.3 ± 1                     | 0.002 |
| Glu at 30 minutes, OGTT (mmol/L)   | 7.6 ± 1.4              | 10.7 ± 2.4                  | <0.001|
| Glu at 60 minutes, OGTT (mmol/L)   | 7.3 ± 2.1              | 12.4                        | <0.001|
| Glu at 90 minutes, OGTT (mmol/L)   | 6.9 ± 1.2              | 11.4 ± 3.8                  | <0.001|
| Glu at 120 minutes, OGTT (mmol/L)  | 6.3 ± 1.1              | 10.2 ± 2.8                  | <0.001|
| Glu at 180 minutes, OGTT (mmol/L)  | 4.6 ± 0.7              | 6.1 ± 1.9                   | 0.044 |
| Insulin at 0 minutes, OGTT (mU/L)  | 12.3 ± 8.3             | 15.7 ± 7.4                  | 0.336 |
| Insulin at 30 minutes, OGTT (mU/L) | 91.8 ± 55.5            | 88.4 ± 59.8                 | 0.687 |
| Insulin at 60 minutes, OGTT (mU/L) | 92.0 ± 70.2            | 114.7 ± 59.6                | 0.204 |
| Insulin at 90 minutes, OGTT (mU/L) | 77.8 ± 59.4            | 109.5 ± 50.7                | 0.016 |
| Insulin at 120 minutes, OGTT (mU/L)| 72.2 ± 51.7            | 110.4 ± 44.9                | 0.016 |
| Insulin at 180 minutes, OGTT (mU/L)| 37.2 ± 49.8            | 54.5 ± 31.5                 | 0.022 |
| C-peptide at 0 minutes, OGTT (pmol/L) | 0.8 ± 0.3            | 1.2 ± 0.4                   | 0.010 |
| C-peptide at 30 minutes, OGTT (pmol/L) | 3.2 ± 1.1            | 3.2 ± 1.2                   | 1.000 |
| C-peptide at 60 minutes, OGTT (pmol/L) | 3.7 ± 1.3            | 4.4 ± 1.1                   | 0.113 |
| C-peptide at 90 minutes, OGTT (pmol/L) | 3.4 ± 1.2            | 4.7 ± 0.9                   | 0.001 |
| C-peptide at 120 minutes (pmol/L)  | 3.3 ± 1.0              | 4.6 ± 0.9                   | 0.005 |
| C-peptide at 180 minutes (pmol/L)  | 1.8 ± 1.3              | 2.9 ± 1.4                   | 0.029 |
| GLP-1 at 0 minutes (pM)            | 5.2 ± 3.2              | 3.1 ± 1.5                   | 0.169 |
| GLP-1 at 120 minutes (pM)          | 5.5 ± 2.7              | 3.3 ± 2.1                   | 0.014 |
| GIP at 0 minutes (pg/mL)           | 80 ± 56.2              | 81.2 ± 25.2                 | 0.243 |
| GIP at 120 minutes (pg/mL)         | 229.1 ± 88.7           | 257.1 ± 99.9                | 0.614 |
| HOMA-B score                       | 148.3 ± 111.6          | 115.5 ± 57.2                | 0.687 |
| MBCI                               | 9.8 ± 4.7              | 7.5 ± 5.2                   | 0.153 |
| QUICKI                             | 0.34 ± 0.04            | 0.32 ± 0.03                  | 0.113 |
| OGIS index (mL/min m²)             | 387 ± 69.5             | 326.6 ± 59.7                | 0.044 |
| IAI                                | 0.02 ± 0.02            | 0.01 ± 0.01                  | 0.113 |
| HOMA-IR score                      | 2.8 ± 1.9              | 4.3 ± 2.4                   | 0.113 |
| Cholesterol (mmol/L)               | 4.9 ± 0.9              | 4.9 ± 1                     | 0.960 |
| HDL (mmol/L)                       | 1.3 ± 0.3              | 1.2 ± 0.3                   | 0.687 |
| LDL (mmol/L)                       | 3.0 ± 0.8              | 3.1 ± 0.8                   | 0.960 |
| TAG (mmol/L)                       | 1.5 ± 1.1              | 1.9 ± 1.4                   | 0.336 |
| SHBG (nmol/L)                      | 26.1 ± 9.6             | 21.7 ± 8.4                  | 0.288 |
| Free testosterone (pmol/L)         | 5 ± 3.4                | 6.7 ± 2.8                   | 0.064 |
| Total testosterone (nmol/L)        | 2.1 ± 1.8              | 1.3 ± 0.8                   | 0.479 |
| Androstenedione (nmol/L)           | 7.1 ± 3.8              | 4.5 ± 2.1                   | 0.347 |
| DHEAS (µmol/L)                     | 6.7 ± 5                | 5.3 ± 2.3                   | 0.650 |
| DXA %fat                           | 44.9 ± 6.8             | 45.8 ± 2.9                  | 0.960 |
| VAT mass (g)                       | 675.3 ± 221.4          | 1013.6 ± 195.1              | 0.001 |
| VAT volume (cm³)                   | 730.1 ± 239.3          | 1098.3 ± 211.7              | 0.001 |
| VAT area (cm²)                     | 140.1 ± 45.9           | 210.2 ± 40.4                | 0.001 |

Values were compared using the Mann–Whitney test.

**Abbreviations:** SD, standard deviation; NGT, normal glucose tolerance; IGT, impaired glucose tolerance; IFG, impaired fasting glucose; BMI, body mass index; Glu, glucose; OGTT, oral glucose tolerance test; GLP-1, glucagon-like peptide-1; GIP, glucose-dependent insulinotropic peptide; HOMA-B, homeostasis model assessment for beta-cell function; MBCI, modified beta-cell function index; QUICKI, quantitative insulin sensitivity check index; OGIS, oral glucose insulin sensitivity; IAI, insulin action index; HOMA-IR, homeostasis model assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TAG, triacylglycerol; SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone sulfate; DXA, dual energy X-ray absorptiometer; VAT, visceral adipose tissue.
between groups of approximately 25% with 80% power, each group had to consist of 13 patients. Patients’ clinical characteristics are shown as mean values with standard deviations. The nonparametric Mann–Whitney test was used to compare continuous variables between different patient groups, while Spearman’s rho was used to assess correlations between different continuous variables. To determine the area under the curve, as well as sensitivity and specificity, receiver operating characteristics curve analysis was used. The cut-off value was determined as the value with the highest sum of sensitivity and specificity. Two-tailed p values of <0.05 were considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics version 21.0 (IBM Corporation, Armonk, NY, USA).

Results

Patients’ baseline characteristics

Twenty-six women were studied. Thirteen of the patients had NGT and 13 had prediabetes. The characteristics of the patients with prediabetes and NGT are shown in Table 1.

Incretin hormones

Baseline plasma GLP-1 levels were not different between the groups. In both groups, GLP-1 levels rose after the glucose load (the change in GLP-1 levels during the OGTT was 0.4 ± 1.4 in the NGT group and 0.2 ± 1.0 in the prediabetes group; p = 0.579). Women in the prediabetes group had significantly lower total GLP-1 levels after the glucose load compared with those in the NGT group (p = 0.014). Receiver operating characteristics curve analysis showed that lower values of GLP-1 at 120 minutes of the OGTT were associated with a higher probability of IGT/IFG (area under the curve = 0.781 [0.600–0.962], p = 0.015) with a cut-off value of 3.02 pM (sensitivity: 0.615, specificity: 0.923).

GIP-1 levels were not different between the groups at baseline. After the glucose load, GIP levels increased without a significant difference between the groups.

Parameters of static and OGTT-related measures of glycemic control

Baseline plasma insulin levels appeared to be higher in the prediabetes group than in the NGT group, but this was not significant. After the glucose load, insulin levels increased in both groups. At 60 minutes, insulin levels appeared to be higher in the prediabetes group than in the NGT group, but this was not significant. However, insulin levels at 90, 120, and 180 minutes during the OGTT were significantly higher in the prediabetes group than in the NGT group (all p<0.05). In both groups, peak plasma insulin levels were reached at 60 minutes of the OGTT (Table 1).

The insulin sensitivity (OGIS) index was significantly lower in the prediabetes group than in the NGT group (p = 0.044). There also appeared to be worse static measures of beta-cell function, as assessed by the HOMA-B, MBCI, and QUICKI, in the prediabetes group than in the NGT group, but these differences were not significant (Table 1). The HOMA-IR and IAI appeared to be higher in the prediabetes group than in the NGT group, but these differences were not significant.

Measures of obesity

There were no significant differences in BMI and waist circumference between the groups. However, women in the prediabetes group had significantly higher visceral adipose tissue mass, volume, and area, as measured by DXA, than did women in the NGT group (all p = 0.001, Table 1).
Correlations

In the total study population, higher plasma GLP-1 levels at 120 minutes of the OGTT were significantly related to a higher OGIS index (Spearman’s rho $r = 0.424$, $p = 0.031$). Patients with higher plasma GLP-1 levels tended to have a lower VAT mass (Spearman’s rho $r = -0.388$, $p = 0.055$), VAT volume (Spearman’s rho $r = -0.390$, $p = 0.054$), and VAT area (Spearman’s rho $r = -0.381$, $p = 0.060$), but these differences did not reach significance. There were no significant associations between a change in GLP-1 levels during 120 minutes of the OGTT and these clinical parameters.

With regard to the family burden of T2D, no differences in GLP-1 levels at 120 minutes of the OGTT were observed if patients had at least one first-degree relative affected by T2D (GLP-1 levels: $5.1 \pm 3.2$ pM) compared with those with no affected relatives (GLP-1 levels: $4.0 \pm 2.2$; $p = 0.363$). However, patients’ GLP-1 levels during 120 minutes of the OGTT were significantly lower if T2D was present among first degree relatives (the change in GLP-1 during the OGTT was $-0.28 \pm 0.91$ pM for patients with affected relatives versus $-0.04 \pm 1.24$ pM for patients with no affected relatives; $p = 0.047$).

Discussion

In our study, the GLP-1 response to oral glucose was reduced in women who had obesity and PCOS with prediabetes compared with women who had obesity and PCOS with NGT, despite comparable BMI, age, and the same disease phenotype. GLP-1 levels $<3.02$ pM at 120 minutes during the OGTT were associated with prediabetes. Plasma GLP-1 levels at 120 minutes were negatively correlated with visceral adipose tissue and positively correlated with the OGIS index. Furthermore, we found a correlation between a change in GLP-1 levels and a family history of at least one first-degree relative affected by T2D.

The sequence of cause and effect regarding the relationship between glucose tolerance status and the GLP-1 response remains uncertain. There is still controversy whether and to what extent reduced GLP-1 release proceeds development of T2D. In our study, we detected a reduction in the GLP-1 response in women who had obesity and PCOS with prediabetes that preceded development of T2D, despite comparable BMI in both groups. In contrast, some other studies have shown that the GLP-1 response is not impaired in the early pathogenesis of T2D, and that impaired GLP-1 release is a phenomenon secondary to T2D. In agreement with our findings, some previous studies reported a reduced GLP-1 release even in individuals with prediabetes.

In addition to different glucose tolerance states, obesity has been identified as independent and additive predictor of an impaired GLP-1 response. An inverse association between the BMI and GLP-1 response has been shown in several studies. Independent of glucose tolerance status, individuals with obesity and overweight have an impaired GLP-1 response of up to 20% compared with individuals with normal weight. Furthermore, an increase in GLP-1 secretion following weight loss has been reported. In our study, women with prediabetes and NGT had different GLP-1 responses, despite a comparable BMI. However, women in the prediabetes group had a greater amount of visceral adipose tissue as measured by DXA compared with women in the NGT group. We found that postload GLP-1 levels were negatively correlated with visceral adipose tissue, despite a comparable BMI in both groups.

Our results indicate that crosstalk between adipose tissue and the incretin...
system could be specifically related to different adipose tissue compartments. Recently, diverse proteomic profiling of the abundant adipocyte secretome showed that dipeptidyl peptidase 4 is a novel adipokine from adipose tissue, in particular from visceral adipose tissue.\(^{36}\) Dipeptidyl peptidase 4 cleaves and inactivates GLP-1. Therefore, our observed correlation between the GLP response and visceral adipose tissue indicates that the reduced GLP-1 response at least partly reflects an increased elimination of GLP-1 in addition to a more likely impaired release of GLP-1.

Additionally, we showed that postload GLP-1 levels were positively related to the insulin sensitivity index (OGIS). The OGIS index was significantly higher in the NGT group compared with the prediabetes group. GLP-1 is an insulin secretagogue and a defective response of beta-cells to GLP-1 may arise from decreased GLP-1 levels. Defective beta-cell responsiveness to GLP-1 and partial inability of beta-cells to secrete insulin in response to stimulation might contribute to a pathogenic association between incretin signaling and impaired glucose homeostasis.\(^{37}\) However, Vilsboll et al. reported that in contrast to GIP, GLP-1 secretion is not thought to be reduced in prediabetes and T2D.\(^{38}\)

Furthermore, we showed a correlation between a change in patients' GLP-1 levels during 120 minutes of the OGTT and a family burden of T2D. Some of the mechanisms behind the different GLP-1 responses in our cohort may include genetic variation in the regulation of GLP-1 synthesis, which has also been observed in some other studies.\(^{10,35,39,40}\) Twin studies have shown that the GLP-1 response has a heritability of up to 67%.\(^{35}\) A correlation between a history of T2D in a first-degree relative and a greater prevalence of insulin secretory defects in a subset of PCOS that would develop either IGT or T2D by 30 years old has also been reported previously.\(^{41}\)

The main strength of our study is that the study population of women who were matched by age, BMI, and disease phenotype was homogenous. The majority of other studies that addressed this issue were performed in groups with different distributions of sex, age, and BMI.\(^{7,9,16–18,20}\) The inconclusiveness related to the timing and to the extent of the impaired GLP-1 response in relation to impairment of glucose metabolism and obesity could be partly contributed to this heterogeneity. Other strengths of our study were inclusion of a population with early impairment of glucose homoeostasis, with exclusion of differences due to varying duration and severity of impaired glucose homeostasis. We also did not include patients with long-term use of metformin or dipeptidyl peptidase 4 inhibitors that could have disturbed the natural pattern of GLP-1 release.

The major limitation of our study was the small sample size, partly at the expense of homogeneity. Furthermore, GLP1 and GIP levels were not measured at 30 to 45 minutes during the OGTT when their peak usually occurs.\(^{42}\) Moreover, a lack of determination of GLP-1 values at all 30-minute intervals did not allow calculation of the area under the curve and easier comparison with previously published major studies performed in adults with diabetes/prediabetes\(^{7,11}\) and PCOS.\(^{18}\) Future larger, prospective studies should be performed on the basis of our preliminary results.

However, in line with our observations, previous studies reported that differences in GLP-1 levels were not apparent in the early period, but only in the late postprandial period at 120 to 240 minutes after meal ingestion.\(^{11,39}\) Additionally, the GLP-1 response to the OGTT may be considered less relevant than the mixed meal test. However, the mixed meal test is more difficult to standardize than the OGTT and the response between individuals appears to be
greater after a solid meal compared with the OGTT.43

Conclusions and perspectives
Our study shows that the GLP-1 response is significantly lower in women with obesity and PCOS with prediabetes than in the women with obesity and PCOS with NGT. Our findings imply that enhancement of the GLP-1 axis by GLP-1 receptor agonists and DPP-4 inhibitors could be considered as an individually tailored strategy in a subset of obese women with PCOS with the highest metabolic risk and expected rapid conversion rate toward T2D.

Declaration of conflicting interest
The authors declare that there is no conflict of interest.

Funding
The study was supported by the Ministry of Health, Republic of Slovenia, Tertiary Care Scientific grant number 20120047 of the University Medical Centre, Ljubljana.

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