Proinsulin serum concentrations in women with polycystic ovary syndrome: a marker of β-cell dysfunction?

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BACKGROUND: The aim of this study was to establish the effect of polycystic ovary syndrome (PCOS) adjusted for adiposity on proinsulin concentrations. METHODS: Ninety-one women with PCOS and 72 normal cycling (NC) women were recruited. A 2 h, 75 g oral glucose tolerance test was performed. Glucose and insulin were measured in each sample. Proinsulin and C-peptide were determined at 0 and 30 min and the fasting proinsulin/insulin ratio (PI/I) was calculated. Insulin sensitivity was estimated by insulin sensitivity index (ISI) composite, and β-cell function was estimated by insulinogenic index. RESULTS: Insulin, proinsulin and C-peptide concentrations were higher in women with PCOS than in NC women ($P < 0.05$). PI/I and insulinogenic index were similar in both groups. Proinsulin concentrations increased with body mass index ($P < 0.05$) only in women with PCOS; therefore, proinsulin concentrations were higher in obese PCOS patients compared with obese control women ($P < 0.05$). Moreover, a positive association between proinsulin concentrations and waist diameter adjusted for C-peptide ($P < 0.05$) and a negative association between proinsulin concentrations and ISI composite values were observed in PCOS patients ($P < 0.05$). CONCLUSIONS: Data suggest that in PCOS patients an elevated proinsulin concentration could reflect insulin resistance more than β-cell dysfunction. However, the elevated concentration of proinsulin in these patients could also result from impaired β-cell function resulting from intra-abdominal obesity independently of insulin resistance.

Key words: β-cell function/polycystic ovary syndrome/proinsulin

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders, affecting 4–8% of women of reproductive age, being characterized by anovulation and hyperandrogenism (Adams *et al*., 1986; Hull, 1987; Zawadzki and Dunaif, 1992; Ehrmann *et al*., 1995; Franks, 1995; Knochenhauer *et al*., 1998). In addition to these reproductive characteristics, women with PCOS carry an increased risk of developing impaired glucose tolerance and type 2 diabetes at an early age (Dunaif *et al*., 1989; Ehrmann *et al*., 1999; Legro *et al*., 1999). Insulin resistance plays a key role in the predisposition to diabetes in PCOS. In addition to a decrease in insulin sensitivity, many patients with PCOS also exhibit reduced β-cell function, in the absence of glucose intolerance or frank diabetes (O'Meara *et al*., 1993; Dunaif and Fingood, 1996).

Previous studies have demonstrated that a high proinsulin/insulin ratio (PI/I) is related to a decline in glucose tolerance (Saad *et al*., 1990; Williams *et al*., 1991; Reaven *et al*., 1993; Wareham *et al*., 1999; Roder *et al*., 1998, 1999). On the other hand, an elevated fasting PI has been reported to be predictive of development of type 2 diabetes in certain at-risk groups and may precede the diagnosis by 5–20 years (Mykkänen *et al*., 1995; Haffner *et al*., 1997; Wareham *et al*., 1999). Therefore, this parameter has been suggested to be a marker of β cell dysfunction.

There are few investigations of proinsulin serum concentrations in women with PCOS. In one study, the proinsulin concentration was assessed in women with PCOS with different insulin levels, establishing that proinsulin concentrations were higher in hyperinsulinaemic women with PCOS compared with those with PCOS who had normal serum insulin concentrations (Conway *et al*., 1993). In another study, proinsulin concentrations were measured only in normal weight women (eight with PCOS and seven healthy women) during a 75 g oral glucose tolerance test (OGTT). No difference in proinsulin concentration was established between the groups (Gama *et al*., 1996).

Recently, in non-diabetic subjects, proinsulin showed significant and univariate associations with percentage body fat, body mass index (BMI) and waist circumference, adjusted

for C-peptide. This observation suggests a possible detrimental role for intra-abdominal obesity on β-cell function which is reflected as elevated concentrations of proinsulin (Hanley *et al*., 2002)

The aim of the present study was to establish the effect of PCOS adjusted for BMI on proinsulin serum concentrations.

Materials and methods

Subjects

Ninety-one women with PCOS were selected for the study from patients attending the Unit of Endocrinology, Department of Medicine, University of Chile. Inclusion criteria were: chronic oligo- or amenorrhoea, hirsutism, plasma testosterone concentration >0.6 ng/ml [or free androgen index (FAI) >5.0], androstenedione >3.0 ng/ml and characteristic ovarian morphology on ultrasound based on the criteria described by Adams *et al*. (1986). All women were amenorrhoeic and anovulatory, as indicated by progesterone measurements and ultrasound examinations. Hyperprolactinaemia, androgen-secreting neoplasm, Cushing's syndrome and attenuated 21-hydroxylase deficiency, as well as thyroid disease, were excluded by appropriate tests.

In addition, 72 normal cycling women (NC) of similar age and BMI acted as the control group. None of these women had taken oral contraceptives or other medication for ⩾6 months before starting the study.

Prior to the study, informed consent was obtained from all subjects. This study was approved by the local ethics committee.

Study protocol

After a 3 day, 300 g carbohydrate diet and an overnight fast of 10 h, both groups of women were admitted to the Clinical Research Centre in the morning (08:30–09:00). A clinical history was obtained and a physical examination was conducted. Anthropometric measurements were performed in all subjects; height was measured to the nearest 0.1 cm using a wall-mounted stadiometer; weight was measured to the nearest 0.1 kg using a hospital balance beam scale. BMI was defined as weight/height^2 (kg/m^2). The waist diameter was measured to the nearest 0.5 cm at the point of narrowing (as viewed from behind) between the umbilicus and xiphoid process.

A 2 h, 75 g OGTT was performed and the subjects were classified according to the World Health Organization (1999) criteria into normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and type 2 diabetic patients. The subjects with IGT (NC: n = 1 and PCOS: n = 7) or type 2 diabetes (NC: n = 0 and PCOS: n = 4) were excluded from the study, in order to avoid the confusing effect of hyperglycaemia.

NC women were studied in the early follicular phase of the menstrual cycle (days 3–7). In the amenorrhoeic patients, the study began whenever feasible.

Serum glucose and insulin were measured before, and 30, 60, 90 and 120 min after, the glucose load. Proinsulin and C-peptide were measured at fasting and at 30 min. Sex hormone-binding globulin (SHBG), testosterone and androstenedione were determined before the glucose load.

Assays

Serum glucose was determined by the glucose oxidase method (Photometric Instrument 4010; Roche, Switzerland). The coefficient of variation (CV) of this method was <2.0%. Serum insulin, C-peptide and testosterone were assayed by radioimmunoassay (DPC, USA), total serum proinsulin was assayed by specific radioimmunoassay (Linco Research Inc., USA), which has cross-reactivities with insulin, C-peptide and des 64, 65 proinsulin of <0.1% and a cross-reactivity with des (31,32) pro-insulin of 95%, and a detection limit of 0.15 pmol/l. SHBG was determined by radioimmunometric assay (DPC). The intra- and inter-assay CV were 5 and 8% for insulin, 3.4 and 7.2% for C-peptide, 9.6 and 8.6% for testosterone, 3.7 and 4.9% for androstenedione, 2.0 and 5.0% for proinsulin and 3.8 and 7.9% for SHBG.

Calculations

To assess β-cell function, we used the insulinogenic index, calculated as the ratio of the increment in the serum insulin concentration to that in the serum glucose concentration during the first 30 min after the ingestion of glucose (Matsumoto *et al*., 1997). Insulin sensitivity from the OGTT was estimated using the index proposed by Matsuda and DeFronzo (1999) [insulin sensitivity index (ISI) composite]. The fasting proinsulin/insulin ratio (PI/I) was calculated.

In PCOS patients, peripheral insulin concentrations do not adequately represent insulin secretion, given that in these women insulin undergoes variable hepatic extraction (Ciampelli *et al*., 1997). Therefore, the association between proinsulin concentrations and waist diameter was adjusted for C-peptide as previously described (Hanley *et al*., 2002).

Statistical analysis

All values are expressed as means and ranges. Differences in continuous data between study groups were analysed using Student's t-test. The Mann–Whitney test was applied when variables were not normally distributed. The association between continuous variables was assessed through correlation analysis and multiple linear regression techniques. The significance level was set at 5%.

Results

Table I shows the clinical and metabolic characteristics of the control and PCOS groups. By design, age and BMI were not different between the groups. Waist diameter, androstenedione, testosterone and FAI were significantly higher and SHBG was significantly lower in women with PCOS compared with NC

Table I. Clinical and metabolic characteristics of normal cycling women (NC) and polycystic ovary syndrome (PCOS) women

	NC (n = 71)	PCOS (n = 80)
Age (years)	24.4 (16.0–36.0)	23.3 (16.0–37.0)
BMI (kg/m^2)	27.1 (19.0–41.9)	28.0 (17.3–38.6)
Waist diameter (cm)	86.1 (65.0–124.0)	86.8 (60.0–119.0)*
SHBG (nmol/l)	61.24 (20.52–138.6)	33.09 (9.94–98.03)*
Testosterone (ng/ml)	0.35 (0.15–0.57)	0.80 (0.61–1.72)*
FAI	1.98 (0.77–4.76)	14.32 (5.0–56.5)*
Androstenedione (ng/ml)	1.66 (0.42–2.70)	3.90 (0.85–6.52)*
Fasting glucose (mg/dl)	80.5 (51.0–109.0)	83.0 (60.0–106.0)
Fasting insulin (pmol/l)	86.0 (17.3–219.4)	115.5 (21.8–345.6)*
Fasting proinsulin (pmol/l)	10.5 (4.1–24.1)	12.3 (3.2–28.7)*
PI/I	0.17 (0.04–0.63)	0.15 (0.01–0.69)
Fasting C-peptide (nmol/l)	0.52 (0.24–1.69)	0.61 (0.17–1.32)*
Insulinogenic index	2.89 (0.21–12.9)	2.66 (0.24–9.0)
ISI composite	6.9 (1.2– 22.0)	4.7 (1.0–16.7)*

Values are means and ranges.
*$P < 0.05$.
BMI = body mass index; SHBG = sex hormone-binding globulin; FAI = free androgen index; PI/I = proinsulin/insulin ratio; ISI = insulin sensitivity index.

women ($P < 0.01$). Insulin, proinsulin and C-peptide fasting concentrations were higher and ISI composite was lower in women with PCOS compared with control women ($P < 0.05$). Fasting glucose concentrations, PI/I and insulinogenic index were not different between the groups.

In NC women, a negative association between PI/I and insulinogenic index was observed ($P = –0.596$; $P < 0.0001$), whereas in women with PCOS, PI/I was less strongly correlated with the insulinogenic index ($P = –0.279$; $P = 0.014$).

Table II shows the metabolic characteristics of NC and PCOS women distributed according to BMI into normal weight (BMI <24.9), overweight (BMI: 25–29.9) and obese (BMI >30.0) women.

Comparing both groups of women, in the normal weight women with PCOS, 2 h insulin was higher compared with the normal weight control group. On the other hand, an increase in C-peptide, 2 h glucose and 2 h insulin concentrations and a decrease in ISI composite were observed in overweight women with PCOS.

In obese patients, fasting insulin and proinsulin were significantly higher compared with obese NC women. However, C-peptide concentration and the PI/I ratio were similar. The differences between insulin concentrations in PCOS and NC obese women could be explained by a lower hepatic extraction of insulin in obese women with PCOS. Thus, the real secretion ratio (PI/I) would be higher in obese women with PCOS.

At 30 min after the glucose load, glucose concentrations, C-peptide and proinsulin were significantly higher in the obese PCOS group compared with the obese women. The insulinogenic index was not different between groups.

In addition, in these obese patients, the 2 h insulin concentrations were higher and ISI composite was lower than in NC women.

Figure 1 shows the correlation between proinsulin concentrations and ISI composite (Figure 1A) and the correlation between proinsulin concentrations and waist diameter (Figure 1B) in PCOS patients and NC women. ISI composite showed a significant negative association with proinsulin concentrations ($P < 0.0001$), whereas waist diameter was positively associated with proinsulin ($P < 0.0001$). After adjustment for C-peptide this latter association was attenuated but remained highly significant ($r = 0.341$; $P = 0.008$).

In NC, no association was observed between these parameters.

Table II. Clinical and metabolic characteristics of normal cycling women (NC) and polycystic ovary syndrome (PCOS) women distributed by body mass index (BMI)

	Normal weight		Overweight		Obese	
	NC ($n = 23$)	PCOS ($n = 30$)	NC ($n = 32$)	PCOS ($n = 18$)	NC ($n = 16$)	PCOS ($n = 32$)
BMI (kg/m^2)	22.3 (19.0–24.8)	22.0 (17.3–24.8)	26.9 (25.0–29.7)	27.6 (25.1–29.3)	33.8 (30.6–41.9)	33.8 (30.1–38.5)
Fasting						
Glucose (mg/dl)	78.0 (51.0–107.0)	79.8 (64.0–95.0)	80.4 (53.0–100.0)	83.5 (67.0–99.0)	84.2 (51.0–109.0)	85.7 (60.0–106.0)
Insulin (pmol/l)	58.4 (19.9–135.9)	66.5 (21.8–178.9)	91.0 (17.3–219.4)	108.2 (46.1–244.3)	115.4 (24.2–216.0)	165.5* (44.7–345.6)
C-peptide (nmol/l)	0.49 (0.21–0.92)	0.46 (0.18–0.82)	0.44 (0.16–0.86)	0.66* (0.28–0.96)	0.72 (0.27–1.69)	0.73 (0.35–1.32)
Proinsulin (pmol/l)	10.7 (4.1–24.1)	10.3 (5.8–20.2)	10.2 (5.3–18.9)	11.4 (3.3–19.6)	10.7 (4.4–18.1)	14.6* (6.2–28.7)
PI/I	0.22 (0.04–0.53)	0.20 (0.06–0.51)	0.15 (0.05–0.51)	0.15 (0.05–0.69)	0.12 (0.04–0.63)	0.10 (0.03–0.34)
30 min						
Glucose (mg/dl)	106.0 (94.0–197.0)	119.5 (85.0–203.0)	119.8 (73.0–200.0)	131.9 (90.0–186.0)	110.1 (70.0–140.0)	132.2* (89.0–218.0)
Insulin (pmol/l)	500.0 (85.0–1242.8)	474.4 (78.4–1177.6)	595.8 (95.28–2096.5)	720.4 (186.6–2163.9)	823.8 (250.3–2670.0)	1112.0 (355.5–2385.7)
C-peptide (nmol/l)	1.99 (0.68–6.59)	2.03 (0.92–3.87)	1.81 (0.25–4.96)	2.48 (0.28–5.38)	1.94 (0.92–5.36)	2.84* (1.10–6.15)
Proinsulin (pmol/l)	34.76 (6.07–83.75)	40.63 (16.47–123.39)	35.71 (10.56–148.13)	41.96 (15.24–80.75)	36.12 (11.58–99.08)	60.54* (17.46–164.56)
Insulinogenic index	2.12 (0.21–8.09)	2.06 (0.25–5.55)	2.89 (0.26–12.99)	2.43 (0.26–9.00)	4.17 (0.63–10.68)	3.70 (0.24–9.00)
120 min						
Glucose (mg/dl)	80.60 (38.0–126.0)	82.54 (42.0–126.0)	85.70 (49.5–139.0)	100.65* (65.0–136.0)	94.13 (50.0–122.0)	93.82 (43.0–135.6)
Insulin (pmol/l)	193.33 (44.2–525.0)	373.6* (25.7–1737.0)	336.33 (89.9–1345.4)	582.5* (97.0–1811.9)	358.50 (97.7–1162.0)	626.4* (126.9–2077.0)
ISI composite	9.1 (3.4–20.7)	7.2 (1.9–16.7)	6.0 (1.1–22.0)	3.7* (1.2–9.6)	5.4 (1.4–20.7)	2.9* (1.0–6.6)

*$P < 0.05$ between NC versus PCOS.
PI/I = proinsulin/insulin ratio; ISI = insulin sensitivity index.

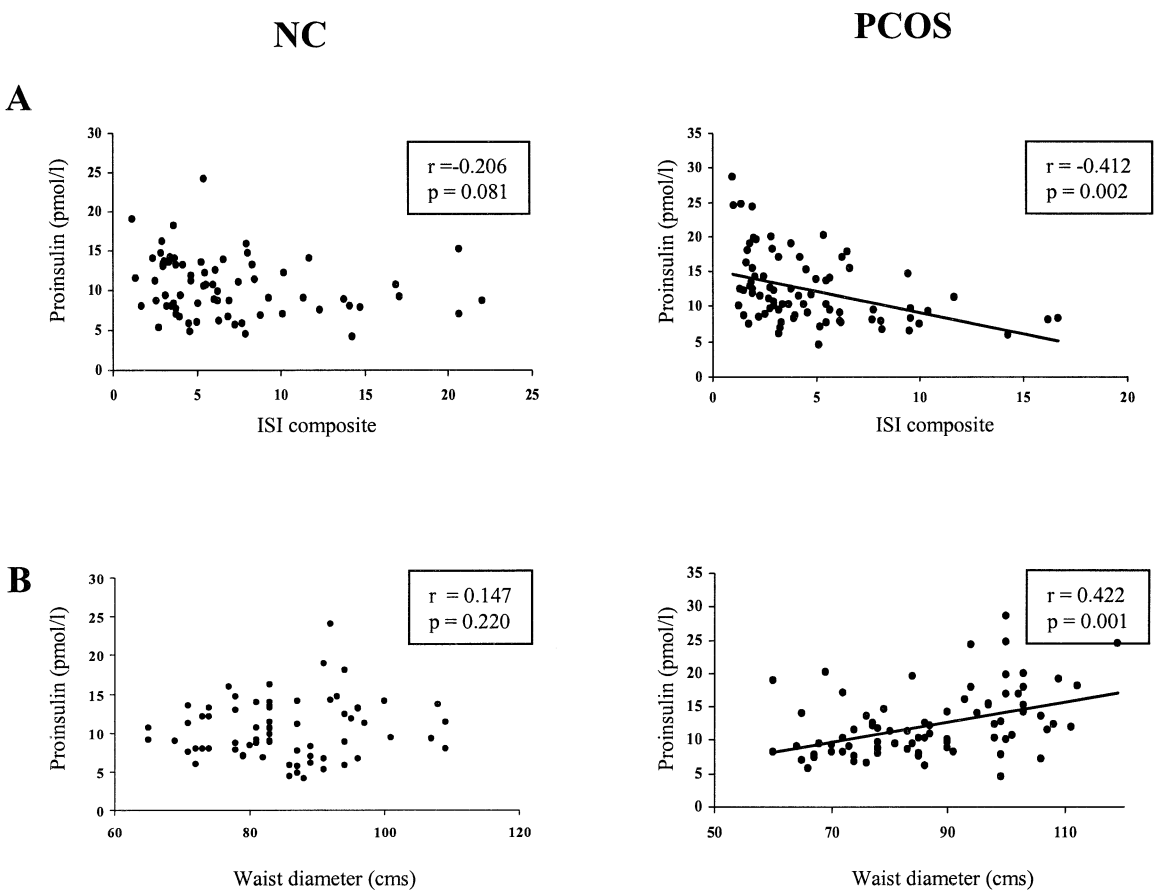

Figure 1. Correlation between proinsulin concentrations and insulin sensitivity index (ISI) composite (**A**) and waist diameter (**B**) in normal cycling (NC) and polycystic ovary syndrome (PCOS) women.

Discussion

In this study, we evaluated proinsulin concentrations in a large sample (n = 151) of NC and women with PCOS with normal glucose tolerance. Metabolic parameters were compared for different BMI groups. Proinsulin concentrations were significantly higher in PCOS patients compared with control women; moreover proinsulin concentrations increased with BMI only in PCOS patients, whereas the PI/I ratio and the insulinogenic index were no different for any of the BMI groups. In PCOS patients, a negative association between proinsulin and ISI composite values and a negative association between proinsulin, adjusted for C-peptide, and waist diameter were observed.

It has been suggested that fasting proinsulin and PI/I could predict the development and progression of type 2 diabetes. However, there are few studies measuring proinsulin concentrations in women with PCOS. According to the present study, in normal weight women, proinsulin concentrations were similar between NC and PCOS women. This observation is in agreement with a previous study which reported no differences in proinsulin concentrations between control women of normal weight and normal weight women with PCOS (Gama *et al*., 1996).

In the aetiology of type 2 diabetes, insulin resistance and β-cell dysfunction are important factors in the development of glucose intolerance (Ferrannini *et al*., 1998). The present study shows that high proinsulin concentrations in women with PCOS with neither IGT nor type 2 diabetes are correlated with a decrease in insulin sensitivity as evaluated by ISI composite, in contrast to normal women. This observation is in agreement with studies in non-diabetic subjects with insulin resistance (Haffner *et al*., 1994; Mykkänen *et al*., 1997) and with a previous report in women with PCOS with different insulin levels in which proinsulin concentrations were higher in hyperinsulinaemic PCOS women compared with those with normal insulin concentrations (Conway *et al*., 1993), suggesting that an increase in proinsulin concentrations in PCOS patients could be a response to elevated insulin resistance.

In the present study, women with PCOS with normal glucose tolerance showed high proinsulin concentrations without an altered PI/I. This phenomenon is probably due to the fact that in these patients, β-cell function was still relatively preserved, similar to that reported recently in obese children and adolescents (Chin *et al*., 2002; Sinha *et al*., 2002). Therefore, insulin concentrations remained elevated and consequently PI/I was not modified, in contrast to other reports where PI/I was evaluated in elderly pre-diabetic subjects (Mykkänen *et al*., 1995). On the other hand, in women with PCOS, independent of BMI, insulin secretion as evaluated by the insulinogenic index was not modified, suggesting again that in these patients high concentrations of proinsulin are probably not a marker of

β-cell dysfunction. However, in both groups of women, PI/I was negatively associated with insulinogenic index, which is a good marker of early β-cell response, since the latter has shown a strong association with insulin secretion evaluated by the hyperglycaemic clamp (Stumvoll *et al*., 2000; Sinha *et al*., 2002). Moreover, the insulinogenic index has been used in epidemiological studies for screening populations at high risk of developing glucose intolerance (Haffner *et al*., 1995; Jensen *et al*., 2002). Therefore, in the women in our study, PI/I could be a useful marker of insulin secretion.

In obese women with PCOS, proinsulin was higher compared with obese control women, indicating that in the PCOS patients, obesity clearly provokes a major demand in β-cell function reflected in hyperproinsulinaemia. However, it is interesting to note that obesity *per se* probably does not explain the increased proinsulin concentrations in women with PCOS, due to the fact that in obese women without PCOS, proinsulin concentrations were not higher than in normal weight women. This observation is in agreement with a previous study, in which proinsulin concentrations were evaluated in obese and non-obese individuals with varying degrees of glucose tolerance (Reaven *et al*., 1993; Roder *et al*., 1999).

In addition, we established a relationship between proinsulin concentration and waist diameter. This latter parameter has been proposed as an indicator of abdominal adiposity and is highly correlated with metabolic alterations associated with the insulin resistance syndrome (Haffner *et al*., 1990). A recent report has suggested that abdominal obesity could have an independent detrimental effect on β-cell function based on the significant association between proinsulin concentrations and waist diameter after adjustment for C-peptide (Hanley *et al*., 2002), supporting the hypothesis that a β-cell dysfunction occurs early in the development of glucose intolerance (Kahn *et al*., 1995; Hanley *et al*., 2002).

In summary, we conclude that in PCOS patients, an elevated proinsulin concentration may reflect both insulin resistance and β-cell dysfunction. Only in PCOS patients was obesity associated with high proinsulin concentrations, suggesting that in these patients, abdominal obesity could alter β-cell function independently of insulin resistance.

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