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Evidence of subpopulations with different levels of insulin resistance in women with polycystic ovary syndrome

Pilar Vigil[1,2,4], Patricio Contreras[2], Jorge L. Alvarado[3], Ana Godoy[1,2], Ana M. Salgado[2] and Manuel E. Cortés[1]

[1]Unidad de Reproducción y Desarrollo, Departamento de Ciencias Fisiológicas, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Alameda B. O'Higgins 340, Santiago, Chile; [2]Fundación Médica San Cristóbal, Luis Pasteur 5292, Vitacura, Santiago, Chile; [3]Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Alameda B. O'Higgins 340, Santiago, Chile

[4]Correspondence address. Tel/Fax: +56-2-6862975; E-mail: pvigil@bio.puc.cl

BACKGROUND: Polycystic ovary syndrome (PCOS) is non-uniformly associated with insulin resistance (IR). We examined IR in women with PCOS. METHODS: Sixty-nine PCOS women were subjected to the insulin suppression test (IST) to determine their steady-state plasma glucose (SSPG) as a direct measure of insulin sensitivity. RESULTS: SSPG exhibited a multimodal distribution suggesting the existence of subpopulations. The heterogeneous distribution of plasma glucose at 180 min ($P = 0.011$), with three modes, suggested differences in the plasma glucose level trajectories during the IST. Hence, the population was separated into three groups: (i) ($n = 33$), subjects with SSPG $\leq$ 152.5 mg/dl, corresponding to the first to fifth deciles; (ii) ($n = 29$), subjects in the interval 152.5 mg/dl < SSPG $\leq$ 300 mg/dl; (iii) ($n = 7$), subjects with SSPG > 300 mg/dl, corresponding to the tenth decile. Plasma glucose distributions at 180 min showed differences in their mean values and ranges among groups ($P < 0.0001$). The trajectories of the groups differed significantly during the IST ($P < 0.0001$). CONCLUSIONS: insulin sensitivity in our patients exhibited a discontinuous distribution, implying that PCOS is a heterogeneous disorder possessing subpopulations regarding IR.

Keywords: hyperandrogenism; insulin resistance; insulin suppression test; polycystic ovary syndrome; subpopulations

Introduction

Polycystic ovary syndrome (PCOS)—the most common endocrine-metabolic disorder among women of reproductive age (Vigil *et al.*, 2005; Rautio, 2006)—has been defined as an ovulatory dysfunction associated with hyperandrogenism, with or without hyperandrogenaemia (Zawadzki and Dunaif, 1992; Biro, 2003; Amato and Simpson, 2004; Vigil *et al.*, 2005). This syndrome shows a prevalence of 5–10% among women of reproductive age (Franks, 1995; Dunaif, 1997; Morin-Papunen, 2000, Benítez *et al.*, 2001; Biro, 2003); also, PCOS has become a recurrent clinical finding among adolescent girls with hyperandrogenism (Nobels and Dewailly, 1992; Vigil *et al.*, 1993; Ibáñez *et al.*, 1996; Apter, 1998; Vigil *et al.*, 1999; Franks, 2002; Biro, 2003), and some characteristics of PCOS can also be found in pre-prepuberal girls (Ibáñez *et al.*, 1998, 2000, 2002). PCOS is characterized by increased secretion of ovarian and adrenal androgens, hyperandrogenic symptoms, such as seborrhoea, acne, hirsutism and alopecia, menstrual irregularity and, in a significant proportion of patients, insulin resistance (IR) (Shoupe *et al.*, 1983; Franks, 1995; Dunaif, 1997; Morin-Papunen, 2000; Dunaif and Thomas, 2001; Vigil *et al.*, 2006). Currently accepted diagnostic criteria are based on a consensus developed at the 1990 National Institute of Child Health and Human Development Consensus Definition (Zawadzki and Dunaif, 1992). Such criteria require the presence of hyperandrogenism and chronic anovulation in the absence of specific diseases of the adrenal gland, ovary or hypophysis that may mimic PCOS, such as non-classical 21-hydroxylase deficiency, hyperprolactinaemia or androgen-secreting tumours (Zawadzki and Dunaif, 1992). However, recent recommendations arising from a conference sponsored by the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine (ESHRE/ASRM) in 2003 suggested that evidence of polycystic ovaries in ultrasonographic scans could also serve as one of the diagnostic criteria for PCOS (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004a,b). Ever since the beginning of the 1980s, there has been evidence of a significant correlation between the levels of androgens and insulin in patients with PCOS (Burghen *et al.*, 1980). In this regard, these patients show a compensatory hyperinsulinaemia caused by the underlying IR.

The fact that IR is not always present in PCOS patients has been widely discussed in a previous review (Contreras, 2003). Even though not all PCOS patients show IR, the prevalence of IR among such patients is remarkable, appearing in 50–70% of the cases (Dunaif, 1997); however, other studies have found a prevalence as high as 76% (Carmina *et al.*, 1992), and yet, another study (del Río *et al.*, 2006) found a much lower prevalence of IR in PCOS women of around 30%. Nevertheless, as mentioned above, although the presence of IR in PCOS populations has been widely described, there have been no specific studies of the magnitude of IR among PCOS women.

The present study was designed with the purpose of examining the prevalence and the differences in magnitude of IR using the insulin suppression test (IST) in a reproductive age population of PCOS patients. This work differs from previous studies in that it included unselected, non-medicated PCOS women, regardless of their body mass index (BMI) or other parameters associated to PCOS.

Materials and Methods

Human subjects approval

The present study protocol was approved by the Fundación Médica San Cristóbal (FMSC) Bioethics Committee. Each subject gave written, informed consent to participate in the study prior to screening.

Subjects

Women ($N = 69$) of reproductive age diagnosed as having PCOS according to the Rotterdam Consensus (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004a,b) were included in the present study. In addition, subjects in the population were screened to rule out disorders that may mimic PCOS, such as non-classical congenital adrenal hyperplasia, thyroid dysfunction or hyperprolactinaemia. Concerning racial characteristics, the subjects were all Hispanic Chilean women. All subjects were tested at the FMSC facilities in Vitacura, Santiago, Chile, where their anthropometric measures—age, height, weight and BMI—and level of fasting glucose were determined. Total testosterone level was measured using an enzyme immunoassay (BioMeriéux) and sex hormone-binding globulin (SHBG) and dehydroepiandrosterone sulphate (DHEA-S) were measured using immunoradiometric assays. On the basis of these measurements, the levels of free testosterone were calculated according to Vermeulen *et al.* (1999).

Octreotide-modified IST

Insulin-mediated glucose disposal was estimated by the IST (Shen *et al.*, 1970; Greenfield *et al.*, 1981; Contreras *et al.*, 1993; Ferrannini and Mari, 1998; Rabasa-Lhoret and Laville, 2001), modified with octreotide, a somatostatin analogue (Pei *et al.*, 1994; McLaughlin *et al.*, 2003). The IST allows the determination of insulin mediated glucose uptake based upon the suppression of endogenous insulin secretion by octreotide and the constant infusion of glucose and exogenous insulin (Pei *et al.*, 1994). The IST is highly correlated ($r = 0.93$) with the euglycaemic-hyperinsulinaemic clamp, the gold standard in the assessment of insulin sensitivity (Greenfield *et al.*, 1981). After overnight fast of 12–14 h, an i.v. catheter was placed in both antecubital veins. One catheter was used for the administration of a 180-min infusion of octreotide (Sandostatin®, 0.27 $\mu g/m^2/h$), insulin (32 mU m^2/min) and glucose (267 mg m^2/min), and the other catheter was used for collecting blood samples. Blood levels were monitored on a Beckman autoanalyser. Blood was initially sampled every 30 min and later on, every 10 min. The average of the last four plasma glucose concentration values (*i.e.* at 150, 160, 170 and 180 min) was calculated for each individual, which is termed the steady-state plasma glucose (SSPG). Previous studies (Greenfield *et al.*, 1981; Pei *et al.*, 1994; Yeni-Komshian *et al.*, 2000) have considered SSPG as evidence for insulin sensitivity, since this value is inversely proportional to insulin sensitivity in the tissues; higher SSPG concentrations, therefore, indicate a more insulin resistant patient (Yeni-Komshian *et al.*, 2000; McLaughlin *et al.*, 2003). The IST has been previously used to assess IR in PCOS women (Cataldo *et al.*, 2006).

Statistical analysis

SSPG data were used to construct frequency distributions and calculation of deciles for the subject population. Data were analysed using descriptive statistics (deciles, mean and SEM), Pearson's correlation coefficient (r) (Sokal and Rohlf, 1981), one-way analysis of variance (one-way ANOVA; Sokal and Rohlf, 1981) and repeated measures multivariate analysis of variance (MANOVA, Sokal and Rohlf, 1981). Proportions were compared by chi-square (χ^2) tests (Sokal and Rohlf, 1981). All statistical analyses were done using SAS Statistical System version 8.2 (SAS Institute Inc., Cary, NC, USA) and Minitab version 12 (Minitab Inc., State College, PA, USA). Significance was determined at $P < 0.05$.

A retrospective study of power of the comparisons showed that due to the magnitude of the differences observed and variability of the subjects, even with a sample size of four individuals per group, our power would have been higher than 80% to detect significance at $\alpha = 0.05$.

Results

Medical examinations prior to the IST showed that the subjects ($N = 69$) had a mean ($\pm$SEM) age of 26.01 $\pm$ 0.76 year (range 14–42 years), BMI of 25.01 $\pm$ 0.54 kg/m^2 (range 18.34–37.88 kg/m^2), fasting glucose 96.38 $\pm$ 1.12 mg/dl (range 75–116 mg/dl) and total testosterone of 2.76 $\pm$ 0.17 nmol/l (range 1.35–6.69 nmol/l). The patient population was separated into deciles of SSPG values (Table 1). SSPG levels showed values similar to those observed in a non-diabetic Caucasian North American population (Yeni-Komshian *et al.*, 2000). The latter population though had a continuous distribution of SSPG in contrast with the discontinuous, multimodal distribution of the parameter observed in our population. A significant correlation was found between BMI and the variables SSPG ($r = 0.41$, $P = 0.00051$) (Fig. 1) and calculated free testosterone ($r = 0.358$, $P = 0.011$) for the general population.

Table 1: Population deciles of SSPG (mg/dl), determined by the IST in women affected by PCOS

Deciles	Decile value	Mean	SEM
1	54.35	43.14	2.66
2	84.35	69.04	3.76
3	110.90	100.25	3.31
4	133.35	121.57	2.73
5	152.50	141.00	2.25
6	180.95	166.63	4.38
7	213.20	196.75	4.71
8	254.40	228.14	4.73
9	290.85	269.71	3.50
10	400.25	346.93	9.91

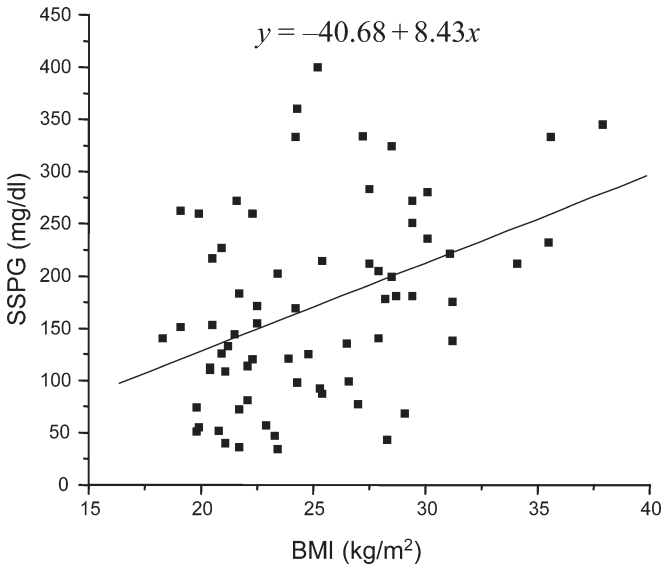

Figure 1: Effect of BMI on SSPG
A significant correlation was found between BMI and SSPG ($n = 60$; $r^2 = 0.168$; $r = 0.410$; $P = 0.00051$)

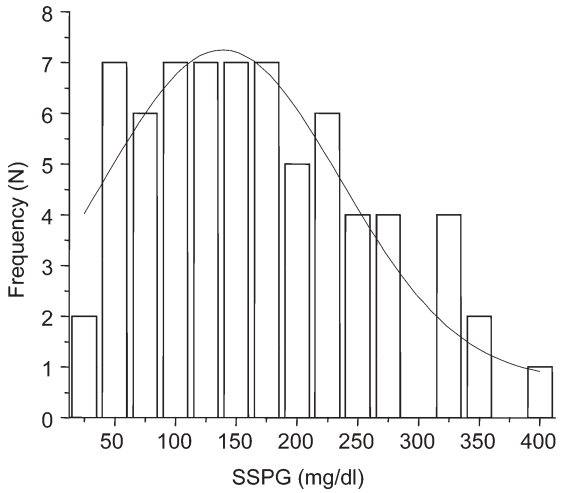

Figure 2: Distribution of the SSPG variable in the PCOS women population ($N = 69$)
A multimodal distribution is observed for the SSPG variable, which is not significantly different in comparison to a normal distribution (Anderson–Darling normality test: $A^2 = 0.717$; $P = 0.059$)

As stated, the variable SSPG had a multimodal distribution (Fig. 2) that was not significantly different from a normal distribution (Anderson–Darling normality test: $A^2 = 0.717$; $P = 0.059$). Multimodality suggested the existence of different groups, or subpopulations, within this population. The distribution of basal plasma glucose (*i.e.* fasting glucose) did not differ from a normal distribution (Anderson–Darling normality test: $A^2 = 4.13$; $P = 0.330$), and it did not show a multimodal but a bimodal distribution (Fig. 3A). Basal plasma glucose levels showed a significant correlation with age ($r = 0.028$, $P = 0.018$). The distribution of plasma glucose at 180 min, however, was significantly different from a normal distribution (Anderson–Darling normality test: $A^2 = 1.015$; $P = 0.011$) and had three well-marked modes (Fig. 3B), suggesting that any differences could be more probably observed in the trajectories of plasma glucose levels during the IST. Based on the distribution of SSPG values in the population, the study subjects were separated into three groups or subpopulations with the following criteria: (i) the first group ($n = 33$) included those subjects whose SSPG was $\leq$152.5 mg/dl (SSPG $\leq$ 152.5 mg/dl), corresponding to the first to fifth deciles; (ii) a second group ($n = 29$) included those whose SSPG was $>$152.5 mg/dl and $\leq$300 mg/dl (152.5 mg/dl $<$ SSPG $\leq$ 300 mg/dl); (iii) a third group ($n = 7$) was formed by those subjects with SSPG $>$300 mg/dl (SSPG $>$ 300 mg/dl), corresponding to the tenth decile of the population. The last two groups were separated due to a well marked discontinuity in the distribution of SSPG values close to 280 mg/dl and the presence of a third mode in the distribution of SSPG values in the original population. The ANOVA on the basal plasma glucose values for the three populations showed non-significant differences among them [$F = 0.94$; degrees of freedom (df) = 2, 66; $P = 0.397$] and the distributions of values showed similar mean values and ranges (Fig. 4A, B, C). Plasma glucose at 180 min showed significant differences among the three groups ($F = 157.25$; df = 2, 66; $P < 0.0001$) and the distribution of values showed marked differences in their mean values and observed ranges (Fig. 4D, E, F). *A posteriori*, Tukey's pairwise comparisons ($\alpha = 0.05$) indicated that all three groups differed. Moreover, the trajectories of the three groups were markedly different showing differences not only in their mean final values, but also in their maxima and the time at which these maxima were observed (Fig. 5). Repeated measures MANOVA indicated that the three group trajectories were different along time (time*group interaction effect: Wilks' $\lambda = 0.1232$; $F = 13.63$; df = 16, 118; $P < 0.0001$).

Age, BMI, fasting glucose, total testosterone, free testosterone, SHBG and DHEA-S were compared among subjects of the different subpopulations in order to examine potential differences between these variables. Of these, only BMI presented significant differences among subpopulations (Table 2). Seventy one percent of the subjects in group 1 had BMI $<$25 kg/m^2, whereas 58% of subjects in groups 2 and 3 had a BMI of 25 kg/m^2 or more (χ^2=6.2706; df = 1; $P = 0.0123$).

Discussion

In 1921, Achard and Thiers described for the first time a relation between hyperandrogenism in women and hyperglycaemia in their classical study "Diabète des femme á barbe" (Achard and Thiers, 1921). Nevertheless, it was only in the early 1980s that IR was linked to PCOS (Burghen *et al.*, 1980; Shoupe *et al.*, 1983; Dunaif *et al.*, 1985). However, according to one of the latest PCOS definitions (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004a,b), IR is not a required condition for the diagnosis of the disorder. Although it is true that IR may represent an important factor in a significant proportion of PCOS women, there are many patients who, although satisfying the diagnostic criteria for PCOS, have insulin sensitivities similar to that of healthy women (Cibula, 2004). On the other hand, obesity is

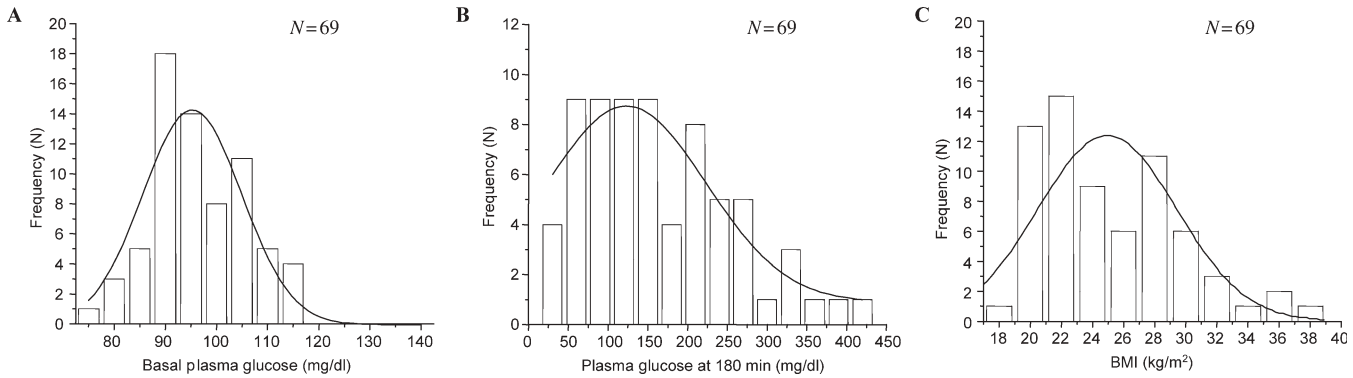

Figure 3: Distribution of the values of (**A**) basal plasma glucose ($N = 69$), (**B**) plasma glucose at 180 min ($N = 69$) and (**C**) BMI ($N = 69$) in the PCOS population

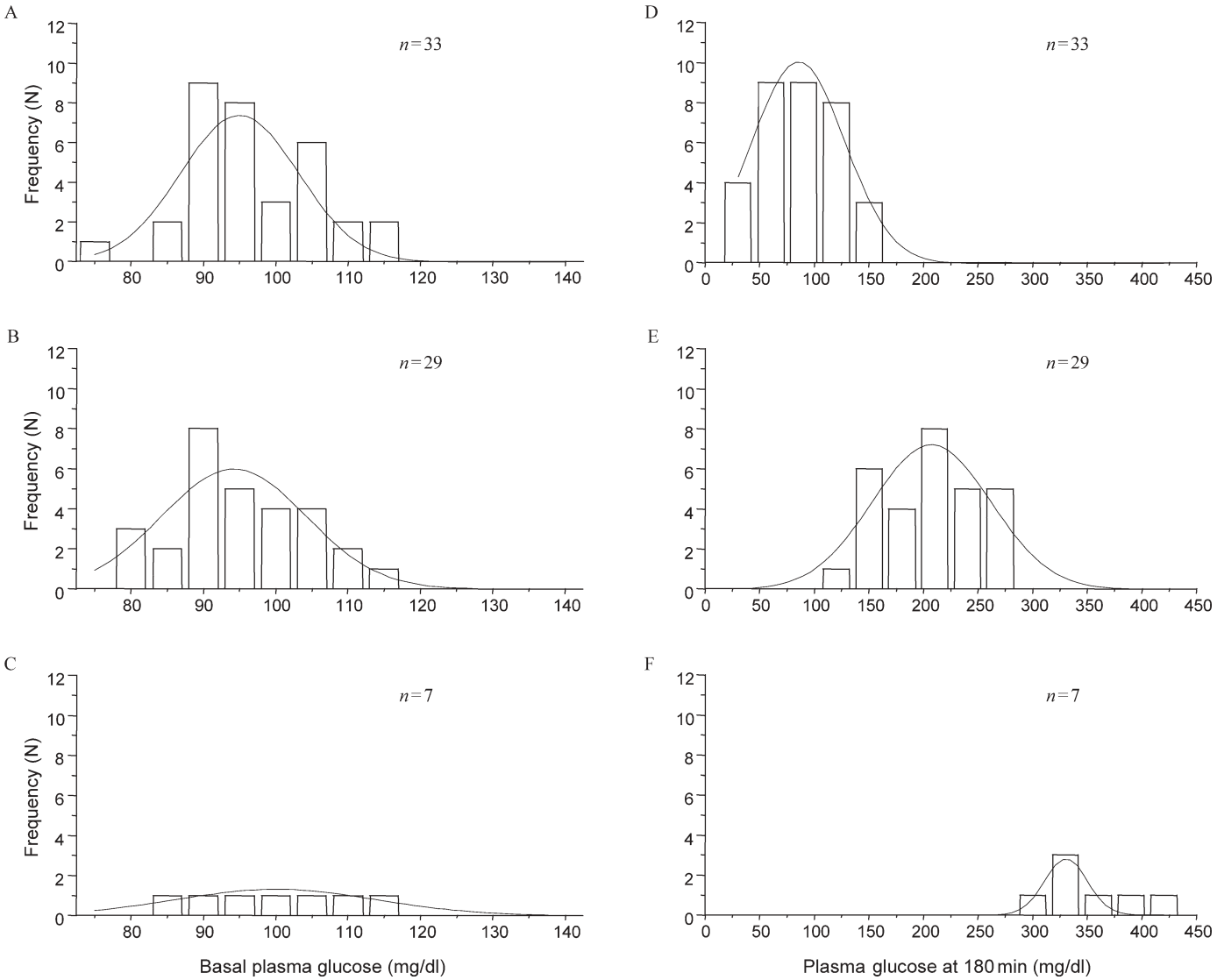

Figure 4: Distribution of the values of basal plasma glucose (**A**–**C**) in the three subpopulations (group 1, $n = 33$; group 2, $n = 29$; group 3, $n = 7$) and of plasma glucose at 180 min (**D**–**F**) for the same groups of patients within the PCOS population

an acquired condition that strongly favours IR and hyperinsulinaemia (Vigil *et al.*, 2005; Rautio, 2006); however, it has to be noted that the presence of these two conditions in patients with PCOS may be independent from obesity and be present in lean women (Chang *et al.*, 1983; Shoupe *et al.*, 1983; Dunaif *et al.*, 1987; Morales *et al.*, 1996). It has also been recognized that both glucose tolerance and insulin sensitivity deteriorates with age (Macut *et al.*, 2002). Despite the fact that our

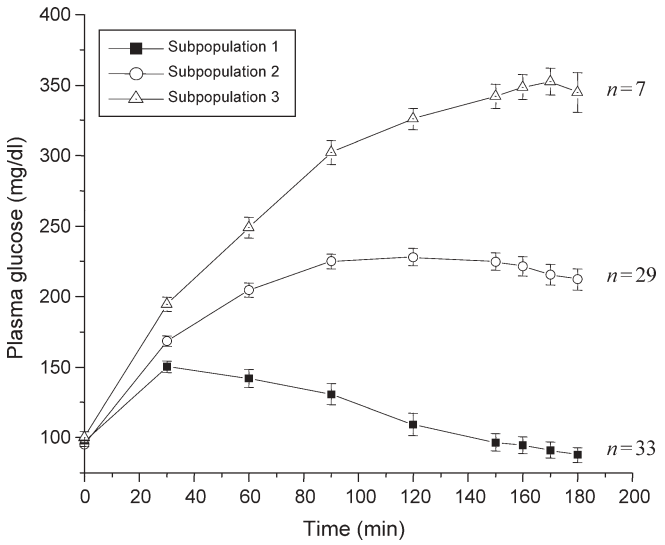

Figure 5: Glucose plasma levels during the IST in the three subpopulations of PCOS patients
Repeated measures multivariate ANOVA indicated that these three curves were different in time (time*group interaction effect: Wilk's $\lambda = 0.1232$; $F = 13.63$; df = 16, 118; $P < 0.0001$)

Table 2: Baseline characteristics of the subjects ($N = 69$) separated into three subpopulations based on their SSPG values

All PCOS subjects	Subpopulation 1	Subpopulation 2	Subpopulation 3
n	33	29	7
Age (year)	25.61 ± 0.97	26.45 ± 1.33	26.14 ± 2.66
BMI (kg/m^2)	23.28 ± 0.56*	25.95 ± 0.87	28.98 ± 2.10
Fasting glucose (mg/dl)	96.82 ± 1.52	94.97 ± 1.81	100.14 ± 3.99
Total testosterone (nmol/l)	2.45 ± 0.25	2.16 ± 0.29	2.58 ± 0.54
Free testosterone (pmol/l)	34.9 ± 5.0	39.6 ± 10.2	42.4 ± 16
SHBG (nmol/l)	58.43 ± 4.63	52.14 ± 9.97	55.92 ± 13.07
DHEA-S (μg/dl)	219.16 ± 17.47	233.91 ± 47.6	196.2 ± 32.73

Values are mean ± SEM.
*Significant differences ($\alpha = 0.05$) between subpopulation 1 and subpopulations 2 and 3, based on Tukey's *a posteriori* pairwise test.

population had a mean age of 26.01 year, and therefore could be classified as a relatively young group of women of reproductive age, a significant relationship between fasting glucose and age was observed. By sampling a young population we reduced the effect of age on IR.

The question posed by this study—namely, the prevalence and magnitude of the IR present in PCOS women—has not been adequately answered to date, mainly due to the lack of an affordable, replicable and straightforward *in vivo* quantification method of insulin sensitivity in clinical practice. The choice of IST was based on the fact that it may be easily carried out in the clinical setting, providing a direct and reproducible insulin sensitivity assessment which is stable over time in individual subjects (Facchini *et al.*, 1999). Moreover, quoting expert opinion on the matter, Ferrannini and Mari (1998), in their comprehensive review on the available methods to measure insulin sensitivity, stated that "the next best choice [to euglycaemic–hyperinsulinaemic clamp] is the somatostatin modification of the insulin suppression test: it is easy and safe and it can be performed at the bed side with minimal training". Our results show that, of 69 studied PCOS patients, 33 of them (47.83%) had a SSPG below 150 mg/dl, which can be considered typical of non-insulin resistant subjects according to ours (Cortés *et al.*, 2006) and previous publications (Yeni-Komshian *et al.*, 2000). These results show that, within this population, a significant fraction of patients is not affected by IR ($n = 33$), which is in agreement with other studies (Cibula, 2004; del Río *et al.*, 2006). Moreover, preliminary analyses on the trajectories of plasma glucose concentrations using non-linear modelling show that at least three types of curves are present in the PCOS population (Fig. 5). The first one (or subpopulation), comprised all the subjects whose SSPG was below 152.5 mg/dl and showed an early peak in plasma glucose during the IST and followed by a marked decrease in plasma glucose that sometimes went below the basal value. A second group, included subjects whose SSPG was between 152.5 and 300 mg/dl, showed an even higher, although delayed, peak in plasma glucose concentration followed by a less marked level decline after the peak. The third group was formed by subjects whose SSPG was higher than 300 mg/dl. This latter group showed an almost continual increase in plasma glucose concentration during the sampling period and had the highest value much later in time. These patterns translated into different trajectories for the groups (Fig. 5) that led to the SSPG differences observed and the multimodal pattern in the SSPG frequency distribution.

The subpopulations showed no differences in terms of age; therefore, the patterns observed might not be attributed to aging effects. More interestingly, the subpopulations did show significant differences in terms of BMI, with the subpopulation 1 (those below 152.5 mg/dl SSPG) showing BMI values significantly lower than those observed in the other two subpopulations. It is important to point out that obesity is associated with IR regardless the presence of PCOS. Besides, it is also known that the percentage of obese women within PCOS populations seems to depend upon geographical distribution (Hoeger, 2001). In the present study, 55.9% of patients presented a BMI of <25 kg/m^2, 30.9% were classified as overweight ($25\ \text{kg/m}^2 \leq \text{BMI} \leq 29.9\ \text{kg/m}^2$) and only 13.2% were classified as obese (BMI ≥ 30 kg/m^2) (Fig. 3C) according to the classification suggested by the National Heart, Lung, and Blood Institute (NHLBI, 1998). It is important to emphasize that obesity and PCOS add their effects and risks (Dunaif *et al.*, 1987; Hoeger, 2001; Vigil *et al.*, 2005) and, as could be expected, in this study the obese PCOS patients presented the highest incidence of IR, but it is important to consider that among patients with normal BMI, IR was also present (Fig. 1).

As has been proposed (Mor *et al.*, 2004), PCOS women with IR could represent a genetically determined subpopulation (or subphenotype). In fact, evidence for a genetic basis of PCOS has been widely reported (Franks *et al.*, 1997; Legro *et al.*, 1998; Amato and Simpson, 2004; Escobar-Morreale *et al.*, 2005; Diamanti-Kandarakis *et al.*, 2006). Recently, a susceptibility gene region for PCOS that regulates adrenal and ovarian

androgen biosynthesis has been located on chromosome 19p13.2 (Urbanek *et al.*, 2005). IR, which can be caused by a post-binding defect in insulin signal transduction (Dunaif, 1997; Morin-Papunen, 2000; Dunaif and Thomas, 2001; Amato and Simpson, 2004), also demonstrates familial aggregation consistent with a genetic trait (Benítez *et al.*, 2001; Dunaif, 2006). Furthermore, IR associated with PCOS also shows an increased incidence among certain ethnic groups such as Mexican-Americans (Goodarzi *et al.*, 2005).

In conclusion, our results showed heterogeneity within the population of PCOS women with respect to IR. These results, although preliminary, could serve as a basis for new genetic studies about IR in PCOS women.

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