Insulin Secretion in Women Who Have Polycystic Ovary Syndrome and Carry the Gly972Arg Variant of Insulin Receptor Substrate-1 in Response to a High-Glycemic or Low-Glycemic Carbohydrate Load

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OBJECTIVE: We evaluated metabolic parameters in Chilean women with polycystic ovary syndrome (PCOS) who were carriers and non-carriers of the glycine-to-arginine substitution at codon 972 (Gly972Arg) variant of insulin receptor substrate-1 and to assess insulin response after oral high- and low-glycemic loads

METHODS: In 146 women with PCOS and 97 healthy women (HW), Gly972Arg genotypes were obtained by polymerase chain reaction, and an oral glucose tolerance test was performed with glucose and insulin measurements. An insulinogenic index, a homeostasis model assessment for insulin resistance, and whole-body insulin sensitivity index (composite) were calculated. Eight carriers and eight non-carriers (four PCOS and four HW, respectively) underwent a 50-g glucose (high glycemic) or 50-g fructose (low glycemic) load with serum glucose and insulin measurements at 15-min intervals for 3 h.

RESULTS: The frequency of the Gly972Arg variant was higher in PCOS patients than in HW ($P < 0.05$). The insulinogenic index was lower in HW carriers than in non-carriers ($P < 0.05$). In PCOS carriers, 2-h insulin was higher than in those without the mutation. In overweight PCOS carriers, the homeostasis model assessment for insulin resistance was higher and the insulin sensitivity index was lower than in PCOS patients without the mutation. In HW carriers, a delay in the maximal response of insulin secretion was observed, with a decrease of 26.7% in insulin concentrations 30 to 60 min after the 50-g glucose load. Glucose concentrations increased by 19.7% between 60 and 120 min. Glucose concentrations between 0 and 120 min were 14.9% higher in PCOS carriers than in non-carriers after the 50-g glucose load.

CONCLUSIONS: In HW, this polymorphism appears to be associated with a decrease in insulin secretion; in PCOS women, this polymorphism interacts with obesity to influence insulin resistance, thus contributing to the pathogenesis of the metabolic component of PCOS.

KEY WORDS: insulin receptor substrate-1, polycystic ovary syndrome, insulin secretion

INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders affecting women of reproductive age and is characterized by irregular menses, chronic anovulation, infertility, and hyperandrogenism.[1–6] In addition, many women with PCOS exhibit peripheral insulin resistance and decreased β-cell function in the absence of glucose intolerance or frank diabetes.[7–10] Al-

though it is currently accepted that insulin resistance plays an important role in the pathogenesis of PCOS,[11–13] the mechanisms of the defects in insulin signaling are probably heterogeneous and not completely known.

Insulin receptor substrate (IRS) proteins are critical to signal transduction in insulin target tissue. IRS-1 is the major cytoplasmic substrate of the insulin receptor and is required for insulin-mediated cellular effects including activations of phosphatidylinositol 3-kinase[14] and p70 S6 kinase[15] and for cellular events leading to mitogenesis.[16] Disruption of IRS-1 in mice causes growth retardation and insulin resistance,[17,18] and islets from knockout mice lacking IRS-1 exhibit a marked secretory defect in response to glucose and arginine,[19] suggesting that IRS-1 may also play an important role in regulating insulin secretion in pancreatic β-cells.

Many polymorphisms have been described in IRS-1, with the most common amino acid change being a glycine to arginine substitution at codon 972 (Gly972Arg),[20] which has an overall

	Healthy ($n = 97$)	PCOS ($n = 146$)
Age (y)	24.0 (15.0–36.0)	22.0 (14.0–38.0)†
BMI (kg/m^2)	24.78 (16.02–43.5)	29.0 (12.62–16.02)†
Waist circumference (cm)	81.0 (59.0–124.0)	88.0 (60.0–145.0)†
Fasting glucose (mg/dL)	79.0 (51.0–103.0)	83.0 (60.0–154.0)†
Fasting insulin (μUI/mL)	9.25 (2.11–31.79)	16.80 (3.16–77.5)
SHBG (nM/L)	53.21 (20.52–95.71)	22.87 (4.64–99.46)†
Androstenedione (ng/mL)	1.66 (0.42–2.70)	3.90 (0.85–9.52)†
Testosterone (ng/mL)	0.32 (0.17–0.55)	0.84 (0.6–1.82)†
FAI	2.47 (0.78–3.86)	11.12 (4.5–49.3)†

* Values are medians (ranges).
† $P < 0.05$, Student's t test.
BMI, body mass index; FAI, free androgen index; PCOS, polycystic
ovary syndrome; SHBG, sex hormone-binding globulin

frequency of 5.8% in the general population and is the most
common variant observed in type 2 diabetes.[21] Transfection stud-
ies indicate that this variant may impair insulin-stimulated signal-
ing, especially along the phosphatidylinositol 3-kinase pathway,
which controls glucose transport,[22,23] and decrease sulfonylurea-
and glucose-stimulated insulin secretions compared with cells that
overexpress wild-type IRS-1.[24] In humans, clinical studies have
associated this genetic variant with decreased insulin sensitivity[25]
and impaired glucose-stimulated insulin secretion.[24,26] In women
with PCOS, this polymorphism has been associated with pheno-
typic features of insulin resistance.[27,28] However, its contribution
to the variation in insulin secretion has not been explored in these
patients.

We evaluated metabolic parameters in Chilean women who had
PCOS and were carriers or non-carriers of the Gly972Arg variant.
To assess insulin secretion in more detail, the insulin responses
after oral high- and low-glycemic loads were determined.

MATERIALS AND METHODS

Subjects

One hundred forty-six unrelated women with PCOS, with an age
range of 15 to 35 y, were consecutively recruited between 2000
and 2002 from among patients attending the Unit of Endocrinol-
ogy and Reproductive Medicine, University of Chile (Santiago,
Chile).

Diagnosis of PCOS was made when subjects had chronic
anovulation, presented hyperandrogenism without other specific
causes of adrenal or pituitary disease, and met the diagnostic
criteria for PCOS according to the National Institutes of Health.[3]

Inclusion criteria for participation were chronic oligo- or am-
enorrhea, hirsutism, serum androstenedione concentration higher
than 3.0 ng/mL, total testosterone concentration higher than 0.6
ng/mL, or a free androgen index score higher than 5.0. All women
were amenorrheic and anovulatory according to progesterone mea-
surements and ultrasound examination. Characteristic ovarian
morphology as detected by ultrasound was not considered an
inclusion criterion. Hyperprolactinemia, androgen-secreting neo-
plasm, Cushing's syndrome, attenuated 21-hydroxylase defi-
ciency, and thyroid disease were excluded by the appropriate tests.

Ninety-seven healthy women (HW), 15 to 35 y old with normal
cycles (Table I), acted as a control group. Each woman had a
history of regular 28- to 32-d menstrual cycles, absence of hirsut-

ism and other manifestations of hyperandrogenism, and absence of
galactorrhea and thyroid dysfunction. They had normal hormonal
status, were not receiving oral contraceptives or any drug therapy
for at least 6 mo before starting the study, and had the antecedent
of a normal term pregnancy with vaginal delivery of a healthy
infant. Because PCOS can be diagnosed even in women with
regular menses,[29] only women with the antecedent of a normal
term pregnancy were selected for the control group, to decrease a
possible misleading effect of an inaccurate disease classification.
The women in the control group were recruited from the same city
area as the patients and with the same socioeconomic status.

PCOS patients and HW were included in the study regardless
of their family history of diabetes in first-degree relatives.

All women had given their written consent to participate in the
study, which was approved by the local ethics committee.

Study Protocol 1

All women were admitted to the Clinical Research Center in the
morning (between 8:30 and 9:00 AM) after an overnight fast of
10 h. A clinical history was obtained, and a physical examination
was conducted. A 75-g oral glucose tolerance test was performed
after at least 3 d of an unrestricted diet that contained 300 g/d of
carbohydrate. The subjects were classified according to World
Health Organization criteria (1999).[30] Serum glucose and insulin
levels were measured before the glucose load and 30, 60, 90, and
120 min after. Sex hormone-binding globulin, testosterone, and
androstenedione were also determined before the glucose load.
The free androgen index (free androgen index = testosterone
[nM/L]/sex hormone-binding globulin [nM/L]) was calculated.

Study Protocol 2

Eight carriers of Gly972Arg (four PCOS and four HW) and eight
non-carriers (four PCOS and four HW) participated in the second
protocol. They were selected from among the PCOS patients and
HW who participated in the first protocol on the basis of age
(carriers: 28.0, range, 24.0–35.0, versus non-carriers: 26.0, range,
22.0–34.0) and body mass index (carriers: 25.6, range, 23.1–37.5,
versus non-carriers: 26.9, range, 19.4–36.1).

After at least 3 d of an unrestricted diet containing 300 g/d of
carbohydrate and an overnight fast of 10 h, these women were
readmitted to the Clinical Research Center in the morning (be-
tween 8:30 and 9:00 AM). Blood samples were collected before and
at 15-min intervals for 3 h after a 50-g glucose (high glycemic) or
50-g fructose (low glycemic) load by using a sampling device that
allowed the continuous withdrawal of blood through a heparinized
catheter as previously described.[31] Serum glucose and insulin
levels were measured in each sample.

Data Analysis

The measurements derived from the oral glucose tolerance test
were: 1) serum fasting glucose, serum fasting insulin, and ho-
meostasis model assessment for insulin resistance (HOMA$_{IR}$) ac-
cording to Matthews et al.[32]; 2) an insulinogenic index (ΔI 0–30
min/ΔG 0–30 min),[33] and an insulin sensitive index (ISI) com-
posite according to Matsuda and DeFronzo[34]; 3) serum levels of
glucose and insulin at 2 h; and 4) areas under the curve for glucose
and insulin.

According to the data obtained from the glucose load, the
frequencies of type 2 diabetes were 0% in HW and 2.4% in PCOS
women. Diabetic PCOS women were excluded from data analysis.

The measurements derived from the oral 50-g glucose or 50-g
fructose load included 1) time of maximal response and 2) areas
under the curve for glucose and insulin at the times of maximal and
minimal responses.

TABLE II.

METABOLIC CHARACTERISTICS OF HEALTHY WOMEN ACCORDING TO THE GLY972ARG VARIANT*

	Gly/Gly ($n = 93$)	Gly/Arg ($n = 4$)	P†
Fasting			
Glucose (mg/dL)	79.0 (51.0–103.0)	85.25 (78.0–89.0)	0.314
Insulin (μUI/mL)	8.51 (2.11–31.79)	11.50 (6.64–13.62)	0.479
HOMA$_{IR}$	1.63 (0.37–7.65)	2.36 (1.38–2.92)	0.369
30 min			
Glucose (mg/dL)	112.0 (69.0–201.0)	152.0 (121.0–197.0)	0.020
Insulin (μUI/mL)	59.18 (7.24–292.20)	41.58 (16.99–115.89)	0.435
Insulinogenic index	1.65 (0.11–10.70)	0.47 (0.10–0.86)	0.014
2 h			
Glucose (mg/dL)	81.0 (58.0–143.0)	109.0 (89.0–126.0)	0.036
Insulin (μUI/mL)	32.79 (12.30–194.99)	26.19 (23.41–58.42)	0.759
AUC glucose (mg/dL per 2 h)	10,980 (5955–17,985)	15,765 (12,892–16,455)	0.008
AUC insulin (μUI/mL per 2 h)	5350 (1528–21,827)	5164 (2618–10,128)	0.927
ISI composite	6.47 (1.16–36.01)	5.42 (3.78–6.32)	0.355

* Values are medians (ranges).
† Uncorrected P values were calculated by the Mann-Whitney test.
Arg, arginine; AUC, area under the curve; Gly, glycine; HOMA$_{IR}$, homeostasis model assessment for insulin resistance; ISI, insulin sensitivity index

Assays

Serum glucose was determined by the glucose oxidase method (Photometric Instrument 4010; Roche, Basel, Switzerland). The coefficient of variation of this method was less than 2.0%. Serum insulin and testosterone were assayed by radioimmunoassay (Diagnostic System Laboratories, Webster, TX, USA), androstenedione was assayed by radioimmunoassay, and sex hormone-binding globulin was assessed by radioimmunometric assay (Diagnostic Products Corp., Los Angeles, CA, USA). The intra- and interassay coefficients of variation were 5% and 8% for insulin, 7.0% and 11.0% for testosterone, 3.7% and 4.9% for androstenedione, and 3.8% and 7.9% for sex hormone-binding globulin, respectively.

Molecular Analysis

The Gly972Arg genotypes were obtained by amplification with polymerase chain reaction using the forward primer 5′-CTT CTG TCA GGT GTC CAT CC-3′, the reverse primer 5′-TGG CGA GGT GTC CAC GTA GC-3′, and BstN-1 restriction enzyme digestion.[35]

Statistical Analysis

Continuous variables are expressed as medians and ranges. Differences in baseline characteristics between the HW and PCOS groups were assessed by Student's t test. Comparisons between carriers and non-carriers were evaluated by the Mann-Whitney test. $P < 0.05$ was considered statistically significant.

RESULTS

Table I lists the clinical and hormonal characteristics of HW and women with PCOS. Body mass index differed significantly between groups. Mean age was younger among PCOS women compared with HW. The introduction of a normal term pregnancy in the inclusion criteria of the control group could explain the age differences between groups. Waist circumference, serum androstenedione concentrations, total serum testosterone concentrations, and free androgen index were significantly higher in the PCOS

group. Moreover, PCOS women had fasting glucose and insulin concentrations significantly higher than control women.

The frequency of the Gly972Arg variant (0.10) was significantly higher ($P < 0.05$) in PCOS patients than in HW (0.04). Genotype frequencies met Hardy-Weinberg expectations in both groups and were similar to those reported previously.[28]

As shown in Table II, fasting values in the HW group did not differ between carriers and non-carriers of the Gly972Arg variant. However, glucose values at 30 and 120 min, area under the glucose curve, and insulinogenic index differed significantly between carriers and non-carriers in the HW group. HOMA$_{IR}$ and ISI composite did not differ between HW carriers and non-carriers of the Gly972Arg variant.

Table III lists the metabolic parameters in diabetes-free PCOS patients according to the Gly972Arg variant. In PCOS women, fasting values did not differ between carriers and non-carriers of the Gly972Arg variant. However, 2-h levels of insulin after stimulation were higher in carriers. HOMA$_{IR}$, ISI composite, and insulinogenic index did not differ between PCOS carriers and non-carriers of the Gly972Arg variant.

The association between Gly972Arg genotype and the insulinogenic index, HOMA$_{IR}$, and ISI composite of overweight (body mass index $\geq$ 25 kg/m^2; World Health Organization criteria) and normal weight (body mass index < 24.9 kg/m^2) in HW carriers of the Gly972Arg variant was examined. The normal-weight subjects showed no differences in these parameters between carriers and non-carriers.

Among the PCOS carriers, nine were overweight and five were normal weight. When overweight PCOS carriers were compared with overweight PCOS non-carriers, HOMA$_{IR}$ was higher (4.57, 1.5–19.8, versus 3.76, 0.86–13.73; $P < 0.05$) and the ISI composite was lower (1.78, 0.69–6.61, versus 2.69, 0.79–3.11; $P < 0.05$) in PCOS carriers. Moreover, 2-h levels of insulin after stimulation remained higher in PCOS carriers (169.78 μUI/mL, 24.71–271.53, versus 81.36 μUI/mL, 13.27–348.95; $P < 0.05$). No significant differences were observed between normal-weight PCOS carriers and non-carriers (HOMA$_{IR}$: 1.79, 1.09–1.89, versus 2.04, 0.63–7.37; ISI composite: 7.07, 4.78–8.2, versus 5.28, 1.45–16.19; and 2-h insulin level after stimulation: 31.10 μUI/mL, 17.38–83.64, versus 45.70 μUI/L, 9.41–251.85).

METABOLIC CHARACTERISTICS OF WOMEN WITH PCOS ACCORDING TO THE GLY972ARG VARIANT*

	Gly/Gly ($n = 129$)	Gly/Arg ($n = 14$)	P†
Fasting			
Glucose (mg/dL)	83.0 (60.0–121.0)	88.0 (68.0–103.0)	0.155
Insulin (μUI/mL)	15.51 (3.16–55.86)	17.52 (5.86–77.5)	0.594
HOMA$_{IR}$	3.38 (0.63–13.73)	3.38 (0.63–17.73)	0.494
30 min			
Glucose (mg/dL)	126.0 (72.0–222.0)	137.5 (90.0–218.0)	0.593
Insulin (μUI/mL)	98.15 (12.13–462.2)	96.36 (43.2–332.5)	0.883
Insulinogenic index	2.10 (0.25–10.77)	2.18 (0.70–4.41)	0.742
2 h			
Glucose (mg/dL)	94.0 (42.0–160.0)	97.5 (62.0–161.0)	0.651
Insulin (μUI/mL)	73.96 (9.41–348.9)	157.76 (17.38–271.53)	0.042
AUC glucose (mg/dL per 2 h)	12,975 (7425–22,185)	14,707 (9225–19,020)	0.176
AUC insulin (μUI/mL per 2 h)	10,065 (1938–40,635)	16,999 (4184–27,166)	0.079
ISI composite	3.03 (0.79–16.19)	2.16 (0.69–8.2)	0.190

* Values are medians (ranges).
† Uncorrected P values were calculated by the Mann-Whitney test.
Arg, arginine; AUC, area under the curve; Gly, glycine; HOMA$_{IR}$, homeostasis model assessment for insulin resistance; ISI, insulin sensitivity index

Responses of insulin and glucose in HW and PCOS women after an oral 50-g glucose or 50-g fructose load according to the Gly972Arg variant are shown in Figures 1 and 2. In HW carriers, a delay in the maximal response of insulin secretion was observed (30 versus 90 min), with a decrease of 26.7% in insulin concentrations 30 to 60 min after the glucose load. This phenomenon was
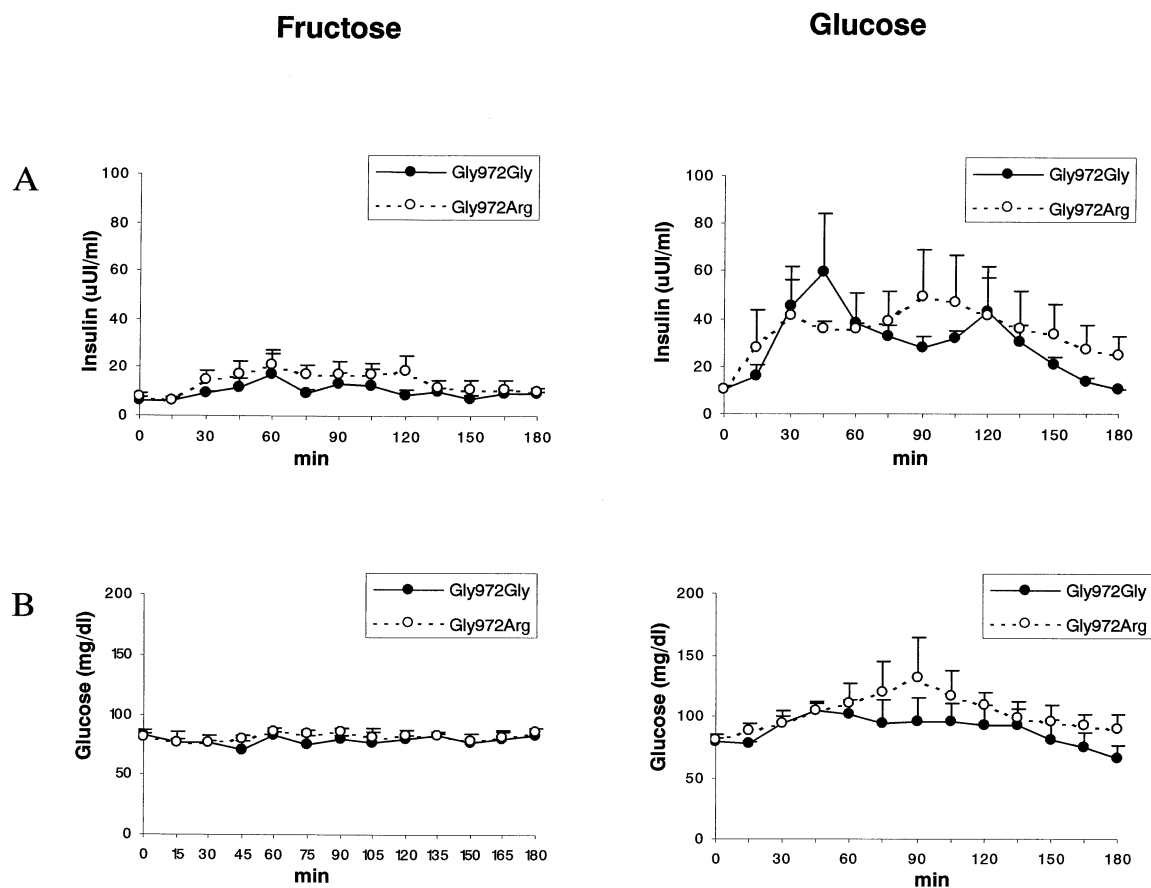
Fructose   **Glucose**

FIG. 1. Mean (± standard error of the mean) responses of insulin (A) and glucose (B) in HW after a 50-g glucose or 50-g fructose oral load according to the glycine-to-arginine substitution at codon 972. HW, health women.
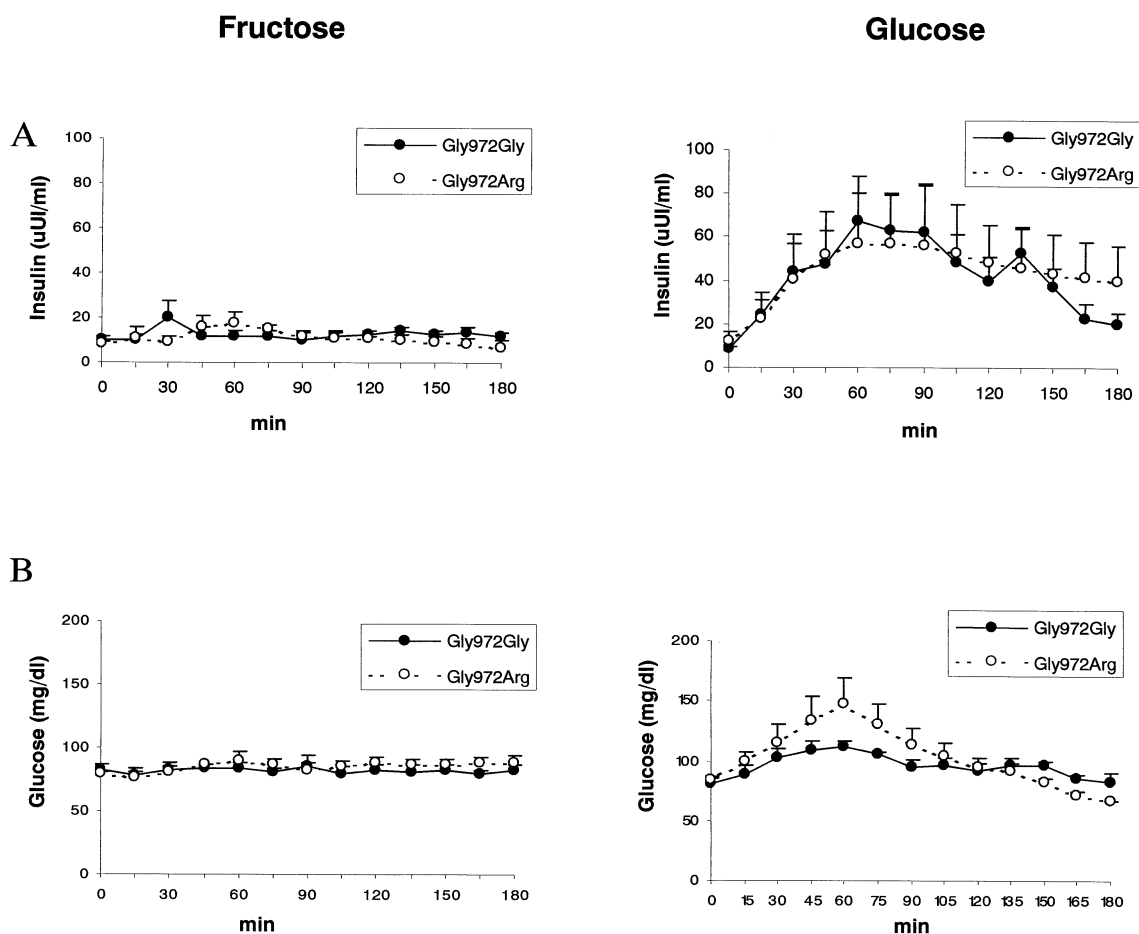
Fructose

Glucose

FIG. 2. Mean (± standard error of the mean) responses of insulin (A) and glucose (B) in women with PCOS after a 50-g glucose or 50-g fructose oral load according to the glycine-to-arginine substitution at codon 972. PCOS, polycystic ovary syndrome.

not observed with the fructose load. Glucose concentrations were similar between HW carriers and non-carriers after the fructose load. However, after the glucose load, HW carriers showed 19.7% higher glucose concentrations between 60 and 120 min compared with HW non-carriers. In PCOS carriers of Gly972Arg, insulin concentrations were similar after the glucose and fructose loads. Glucose concentrations between 0 and 120 min in PCOS carriers were 14.9% higher than those in PCOS non-carriers.

DISCUSSION

In the present study, we examined the relation of the IRS-1 Gly972Arg polymorphism with metabolic features in Chilean women with PCOS and HW. We found that the frequency of the Gly972Arg variant was higher in PCOS patients than in HW. In HW, the Arg allele was associated with a decreased insulin secretion in response to glucose but not with the ISI. In women with PCOS, no clear association of this variant with insulin secretion was observed. However, in these women, the IRS-1 variant seemed to interact with obesity to influence insulin resistance.

The pathogenesis of the metabolic component of PCOS involves a combination of insulin resistance and impaired insulin secretion, similar to that proposed for type 2 diabetes. In vitro and in vivo studies have suggested that the IRS-1 Gly972Arg polymorphism represents a genetic variant that unifies insulin resistance and β-cell dysfunction; therefore, it may be involved in the pathogenesis of the metabolic component of PCOS

Several studies have evaluated the association of the IRS-1 Gly972Arg polymorphism with phenotypic features of PCOS. In the first study,[27] PCOS patients were stratified on the basis of insulin resistance. The IRS-1 Gly972Arg variant was more prevalent in insulin-resistant PCOS patients than in non–insulin-resistant patients or control subjects. Moreover, a gene-dosage effect was found on fasting insulin and $HOMA_{IR}$, suggesting that this variant may have a functional affect on the insulin resistance component of PCOS. This observation is in agreement with our previous study[28] and the data of the present study, in which the IRS-1 variant interacts with obesity to influence insulin resistance in PCOS patients, but has little effect on normal weight subjects, as previously suggested.[25,35] These results clearly contrast with the those of one report, in which no association of the IRS-1 genotype with any clinical or hormonal parameters in non-diabetic white and black PCOS patients was found.[36]

In HW with the Gly972Arg polymorphism, we found lower insulinogenic index values with normal total insulin secretion (expressed as area under the curve) during an oral glucose tolerance test, thus suggesting a decrease in early-phase insulin secretion in these women. In PCOS women, a similar phenomenon was observed, although it was not statistically significantly. The postprandial responses after a high-glycemic or a low-glycemic carbohydrate load were concordant with these observations and with the notion that this polymorphism may impair early-phase insulin secretion, as previously described in diabetic patients[37] and normal glucose tolerance subjects when using more sophisticated meth-

ods.[26] Nevertheless, our approach based on postprandial responses to different carbohydrates is a simple method to evaluate gene–nutrition interactions and probably to establish some dietary recommendations for carriers of the IRS-1 Gly972Arg polymorphism.

Even though the mechanism underlying this effect cannot be inferred from the present study, in vitro studies have suggested that this variant impairs the ability of insulin to activate the phosphatidylinositol 3-kinase/Akt/GSK-3 signaling pathway, leading to defects in glucose transport[23] and in the insulin secretory process in pancreatic β-cells.[24] Therefore, these in vitro studies may explain the functional significance of the phenomenon observed in carriers of the Gly972Arg polymorphism in the present study, in which an early-phase decrease in insulin secretion was followed by an increase of glucose concentrations.

In conclusion, the Gly972Arg polymorphism in IRS-1 in HW appears to be associated with decreased insulin secretion, and this polymorphism interacts with obesity to influence insulin resistance in women with PCOS. This polymorphism may contribute to the pathogenesis of the metabolic component of PCOS, at least in the development of insulin resistance. Further studies are needed to evaluate β-cell function with more accurate methods in PCOS patients.

ACKNOWLEDGMENTS

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