Metabolic Effects of the Gly1057Asp Polymorphism in *IRS-2* and Interactions With Obesity

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Insulin receptor substrate (IRS)-2 plays an important role in insulin signaling and its disruption results in diabetes in mice. In humans, the *IRS-2* Gly1057Asp substitution was associated with lower risk of type 2 diabetes in lean individuals, but with a higher risk in obese individuals. To clarify the role of *IRS-2* on the development of type 2 diabetes and obesity in Pima Indians, and particularly to investigate whether the effects of the Gly1057Asp polymorphism on metabolism are mediated by obesity, molecular scanning of the gene for mutations was performed and interaction of the polymorphism with obesity was tested. We identified the previously described Gly1057Asp mutation as well as a rare Asp819His mutation and four silent polymorphisms. The effect of the Gly1057Asp mutation on type 2 diabetes and obesity was tested in a large cohort of Pima Indians (n = 998). A subgroup of nondiabetic full-heritage Pima Indians (n = 233) had measurements of body composition, glucose tolerance, insulin action (M), endogenous glucose production (EGP; hyperinsulinemic clamp), acute insulin response (AIR, 25-g intravenous glucose tolerance test, n = 118 normal glucose-tolerant subjects), and percutaneous fat biopsy specimens from the periumbilical region (n = 160). A total of 132 nondiabetic subjects were included in longitudinal analyses. The frequency of the Asp1057 allele was 0.6. In cross-sectional analyses, subjects homozygous for the Asp1057 allele (Asp/Asp) had a higher prevalence of type 2 diabetes than heterozygote individuals and subjects homozygous for the Gly1057 allele (X/Gly, P = 0.04). There was no effect on BMI (P = 0.78) or gene-BMI interaction on the prevalence of type 2 diabetes (P = 0.57). In the nondiabetic subgroup, subjects with Asp/Asp had higher percent body fat (P = 0.01), BMI (P = 0.02), and waist circumference (P = 0.004), but there was no difference in metabolic characteristics (all P > 0.2). However, the relationship between percent body fat and fasting glucose, basal EGP, EGP during the clamp, AIR, and subcutaneous abdominal adipocyte size was significantly different in the Asp/Asp group (P for interaction = 0.02, 0.06, 0.0007, 0.08, and 0.006, respectively) compared with the X/Gly group, suggesting a more detrimental effect of Asp homozygosity on these traits with increasing percent body fat. In longitudinal analyses, among subjects in the upper tertile of change in percent body fat, those with Asp/Asp had a larger increase in fasting and postprandial glycemia and basal EGP and a larger decrease in M and AIR than subjects with X/Gly, independent of change in obesity (all P < 0.05). In conclusion, our findings suggest that the association of homozygosity for the Asp1057 allele in *IRS-2* with type 2 diabetes in Pima Indians may be mediated by interaction of the polymorphism with obesity on several diabetes-related traits. *Diabetes* 52:1544–1550, 2003

Insulin receptor substrate (IRS)-2 plays an important role in insulin signaling. Together with IRS-1, it mediates most insulin effects, especially those associated with somatic growth and carbohydrate metabolism (1). Disruption of *IRS-2* was shown to cause type 2 diabetes in mice (2). This result could be attributed largely to hepatic insulin resistance and lack of β-cell compensation (2–4). In humans, a number of polymorphisms have been identified in the *IRS-2* gene. Among those, the amino acid substitution Gly1057Asp was found in various populations with a prevalence sufficiently high to modulate a population's risk of type 2 diabetes. In Caucasians, Finns, and Chinese, however, this variant was not associated with type 2 diabetes (5,6). Although the polymorphism was associated with decreased insulin sensitivity and impaired glucose tolerance in women with polycystic ovary syndrome (7), it showed no association with insulin sensitivity in other studies (6,8,9). In contrast, another study in women with polycystic ovary syndrome found that homozygous carriers of the Gly1057 allele had higher 2-h plasma glucose concentrations during an oral glucose tolerance test (OGTT) (10). Decreased serum insulin and C-peptide concentrations during an OGTT were reported in middle-aged glucose-tolerant Danish males carrying the Asp1057 allele (9). However, using formal β-cell function tests, associations with insulin secretion were not reproduced in German, Finnish, and Swedish populations (6,8,9).

In an Italian population, the effect of the Asp1057 allele on the risk of type 2 diabetes depended on the presence or the absence of obesity. Lean subjects with the Asp allele had a decreased risk while obese subjects had an in-

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AIR, acute insulin response; AUC, area under the curve; DEXA, dual-energy X-ray absorptiometry; EGP, endogenous glucose production; EMBS, estimated metabolic body size (fat-free mass + 17.7 kg); IRS, insulin receptor substrate; M, insulin-stimulated glucose disposal; OGTT, oral glucose tolerance test; SAAS, subcutaneous abdominal adipocyte size; SNP, single nucleotide polymorphism.

creased risk (11). To test whether this polymorphism has similar effects in Pima Indians, a population with one of the highest reported prevalence and incidence rates of obesity and type 2 diabetes, we investigated first whether it is associated with obesity and type 2 diabetes in this Native American population, and second, whether it has interactions with obesity on diabetes status and/or on metabolic characteristics. In addition, the entire gene was screened for other potentially relevant variants.

RESEARCH DESIGN AND METHODS

Subjects

Associations of the Gly1057Asp polymorphism with type 2 diabetes and obesity. A total of 998 Pima Indians who are participants in ongoing studies of the pathogenesis of obesity and type 2 diabetes were included in this analysis. Diabetes status was determined by a 75-g OGTT, and the results were interpreted according to the World Health Organization 1985 criteria (12).

Associations of the Gly1057Asp polymorphism with anthropometrics and metabolic characteristics: cross-sectional analyses. Healthy nonsmoker, nondiabetic full-heritage Pima Indians (n = 233) between 18 and 50 years of age were admitted to the National Institutes of Health (NIH) Clinical Research Unit in Phoenix, Arizona, where they were given a weight-maintaining diet (50% of calories as carbohydrate, 30% as fat, and 20% as protein) and abstained from strenuous exercise. After at least 3 days on the diet, subjects underwent a mixed meal test, assessment of body composition, glucose tolerance, insulin sensitivity, endogenous glucose production (EGP), and acute insulin response (AIR; n = 118, only normal glucose-tolerant subjects) and had measurements of subcutaneous abdominal adipocyte size (n = 160).

Associations of the Gly1057Asp polymorphism with anthropometrics and metabolic characteristics: longitudinal analyses. Longitudinal data were obtained from a subgroup of 132 nondiabetic subjects who underwent a mixed meal test, had measurements of anthropometrics, glucose tolerance, AIR, and insulin action, and were nondiabetic at the follow-up visit. For the analyses they were divided into tertiles according to change in percent body fat. The protocol was approved by the Tribal Council of the Gila River Indian Community and by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases, and all subjects provided written informed consent before participation.

Methods

Body composition. Body composition was estimated by underwater weighing with determination of residual lung volume by helium dilution (13) or by total body dual-energy X-ray absorptiometry (DEXA) (DPX-L; Lunar, Madison, WI) (14,15). Percent body fat, fat mass, and fat-free mass were calculated as previously described (16), and a conversion equation (15) was used to make measurements comparable between the two methods. Waist circumference was measured at the umbilicus in the supine position.

OGTT. After a 12-h overnight fast, subjects underwent a 2-h 75-g OGTT. Plasma glucose concentrations were determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA). Plasma insulin concentrations before 1987 were determined by the Herbert modification (17) of the manual radioimmunoassay of Yalow and Berson (18), before 1998 using an automated radioimmunoassay analyzer (Concept 4; INCBiomedicals, Horsham, PA) and currently by an automated chemiluminescent assay (Access; Beckman Coulter, Fullerton, CA). The mean interassay coefficients of variation for plasma insulin concentrations were 7, 12, and 4% for the three methods. Insulin concentrations measured using the Concept 4 and Access methods were made comparable to the manual method by an algorithm established on the basis of 542 samples (Concept 4 vs. manual) and 250 samples (Access vs. Concept 4). Plasma free fatty acid concentrations were drawn with prechilled syringes and measured according to the method of Miles et al. (19).

Mixed meal test. After a 12-h overnight fast, subjects underwent a standardized test meal containing 35% of their calculated 24-h energy requirements distributed as 40% of total calories from fat, 40% from carbohydrate, and 20% from protein. All subjects finished the meal within 15 min. Blood samples for determination of plasma glucose and insulin concentrations were drawn at 0, 30, 60, 90, 120, 150, 180, 210, and 240 min. The area under the curve (AUC) for glucose and insulin was determined by the trapezoidal method.

Two-step hyperinsulinemic-euglycemic glucose clamp. Insulin action (M) was assessed at physiologic and supraphysiologic insulin concentrations using a two-step hyperinsulinemic-euglycemic glucose clamp, and EGP was determined using a primed (30 μCi) continuous (0.3 μCi/min) 3-[^{3}H]glucose infusion as previously described (20).

Intravenous glucose tolerance test. Insulin secretory response to glucose was measured in response to a 25-g intravenous glucose bolus. The acute insulin response (AIR) was calculated as the mean increment in plasma insulin concentrations above basal in samples obtained 3, 4, and 5 min after the injection of glucose and was adjusted for the mean plasma glucose concentrations calculated from 3, 4, and 5 min.

Fat biopsy and in vitro characterization of adipocytes. In a subgroup of 160 nondiabetic subjects, data on abdominal subcutaneous adipose cell size was available. The procedures for fat biopsies and assessment of adipocyte size have been previously described in detail (21–23).

Screening of the *IRS-2* gene. The *IRS-2* gene was screened for mutations using single-stranded conformational polymorphism (SSCP) and by direct sequencing in 50 Pima Indians as previously described (24). Genomic DNA was amplified by PCR. PCR products were designed to encompass the entire coding region for exons 1 and 2, intron-exon splice junctions, and 461 bp of the 3′ untranslated region.

Genotyping of the Gly1057Asp and Asp819His polymorphisms in IRS-2. Genotyping of the Gly1057Asp polymorphism was done using pyrosequencing (Pyrosequencing, Uppsala, Sweden). The pyrosequencing reaction was amplified on a GeneAmp PCR system 9700 (95°C for 10 min, 95°C for 30 s, 60°C for 1 min, 72°C for 1 min for 38 cycles and 72°C for 10 min) using the forward primer 5′ CAA AAG CCA TCT CGG TGT AGT 3′, the biotinylated reverse primer 5′ GCT CTC CGA CTA CAT GAA CCT C 3′, and the sequencing primer 5′ CGA GGA CAA CGA TGA GGC GGC 3′. The reaction was analyzed on PSQ96 sequencer (Pyrosequencing). Genotyping of the Asp819His variant, which was first identified by screening of the IRS-2 gene, was done using direct sequencing. Sequencing was performed using the Big Dye Terminator Kit (Applied Biosystems) on an automated DNA capillary sequencer (model 3700; Applied Biosystems). Sequences of the oligonucleotide primers used for variant screening were as follows: forward 5′ GAT GTA CTC GCC GGG GCT CT 3′ and reverse 5′ ACT TCT TCT CCG CAG CCC TG 3′. The PCR was amplified on a GeneAmp PCR system 9700 (95°C for 10 min, 95°C for 30 s, 63°C for 1 min, 72°C for 1 min for 35 cycles and 72°C for 10 min).

Statistical analyses. Statistical analyses were performed using the software of the SAS Institute (Cary, NC). Results are presented as mean ± SE. Fasting plasma insulin concentrations, M-low, and AIR were logarithmically transformed to approximate a normal distribution. Because of the relatively low frequency of the Gly1057 allele, subjects who were homozygous for this allele were combined with heterozygotes (X/Gly) for comparison with those subjects homozygous for the Asp1057 allele (Asp/Asp).

The association of the genotype with diabetes was assessed by logistic regression analyses. In these analyses the relationship of an indicator variable for genotype with diabetes status was assessed after adjustment for age, sex, BMI, date of birth, and degree of Pima Indian heritage. A modification of the approach by Abecasis et al. (25) was used to partition the association between or within sibship components. Thus, the models included a term for the sibship mean of the genotypic variable and a term for each individual's deviation from the sibship mean. The former term represents the between-family association (which is potentially influenced by population stratification), and the latter term represents the within-family association (which assesses joint linkage of association and, thus, is robust to confounding by population stratification). These models were fit with generalized estimating equations that account for residual resemblance among siblings (26). Differences in anthropometrics and metabolic characteristics between genotypes were also tested using generalized estimating equation regression models. In these models, body composition, plasma insulin, free fatty acids, plasma glucose, M, EGP, AIR, and subcutaneous abdominal adipocyte size (SAAS) were the dependent variables, whereas age, sex, and genotype (Asp/Asp and X/Gly) were the independent variables.

Because only a limited number of sibships had more than two siblings available for analyses of these metabolic characteristics, and since the ability to partition the associations into between- and within-family components depends on availability of more than two siblings, no attempt was made to evaluate the specific components in these analyses, which means that these analyses are potentially confounded by population stratification.

In longitudinal analyses, changes in anthropometrics and metabolic characteristics (values at the follow-up visit adjusted for values at the initial visit) were adjusted for age at the follow-up visit, time of follow-up, sex, and change in the other covariates. A P value <0.05 was considered to be statistically significant.

RESULTS

Genetic screening. Screening of the *IRS-2* gene for sequence variants revealed six single nucleotide polymorphisms (SNPs). Two of them result in amino acid substitutions, Asp (*G*AC) to His (*C*AC) at codon 819 (Asp819His; allele frequency of C = 0.009) and Gly (G*G*C) to Asp (G*A*C)

TABLE 1
Anthropometrics and metabolic characteristics of Pima Indians who were genotyped for Gly1057Asp in *IRS-2* and underwent metabolic testing (cross-sectional analyses)

	X/Gly [n = 148 (89M/59F)]	Asp/Asp [n = 85 (52M/33F)]	P*
Age (years)	28 ± 1	30 ± 1	0.04
BMI (kg/m^2)	35 ± 1	37 ± 1	0.02
Body fat (%)	34 ± 1	36 ± 1	0.01
Waist circumference (cm)	109 ± 3	117 ± 3	0.004
Fasting glucose (mmol/l)	5.03 ± 0.05	5.01 ± 0.07	0.75
2-h glucose (mmol/l)	7.14 ± 0.15	7.26 ± 0.19	0.62
Fasting insulin (mmol/l)	264 ± 22	288 ± 17	0.68
2-h insulin (pmol/l)	1,422 ± 89	1,542 ± 132	0.97
Glucose AUC$_{\text{meal test}}$ (mmol/l · 240 min)	1,353 ± 12	1,369 ± 20	0.82
Insulin AUC$_{\text{meal test}}$ (pmol/l · 240 min)†	181,920 ± 10,086	208,332 ± 14,052	0.30
EGP$_{\text{basal}}$ (mg/kg · EMBS^{-1} · min^{-1})	1.97 ± 0.02	2.01 ± 0.02	0.31
EGP$_{\text{insulin}}$ (mg/kg · EMBS^{-1} · min^{-1})	0.41 ± 0.03	0.52 ± 0.04	0.22
M-low (mg/kg · EMBS^{-1} · min^{-1})	2.46 ± 0.07	2.30 ± 0.06	0.99
M-high (mg/kg · EMBS^{-1} · min^{-1})	8.44 ± 0.17	7.88 ± 0.22	0.30
AIR (pmol/l)‡	1542 ± 126	1884 ± 198	0.22

Values are mean ± SE. *P for statistical differences between the groups; †available only in n = 200; ‡normal glucose-tolerant subjects (n = 118). In multiple regression analyses, anthropometrics were adjusted for age and sex. Glucose, insulin concentrations, and *M* values were adjusted for age, sex, waist circumference, and percent body fat. EGP and AIR were additionally adjusted for *M*-low. EMBS = fat-free mass + 17.7 kg.

at codon 1,057 (Gly1057Asp; allele frequency of A = 0.6). The remaining SNPs were silent substitutions at codon 723 (Ser: AG*T*→AG*C*; allele frequency of C = 0.22), at 816 (Cys: TG*C*→TG*T*; allele frequency of T = 0.22), at 829 (Pro: CC*C*→CC*T*; allele frequency of T = 0.11) and 891 (Thr: AC*G*→AC*C*; allele frequency of C = 0.15). The Gly1057Asp polymorphism was further investigated for associations with type 2 diabetes and prediabetic subphenotypes.

Associations with type 2 diabetes and BMI: cross-sectional analyses. The genotype distributions were in Hardy-Weinberg equilibrium for the Gly1057Asp polymorphism (P = 0.91, χ^2 test), but not for the Asp819His polymorphism (P = 0.05, χ^2 test, less subjects were homozygous for the His819 allele than expected). For the Gly1057Asp polymorphism, heterozygotes and those subjects who were homozygous for the less common allele (Gly1057 allele) were combined for the analyses. Family-based analyses showed that subjects with Asp/Asp at position 1,057 had a higher prevalence of type 2 diabetes than X/Gly [odds ratio 1.5 (95% CI 1.02–2.29), P = 0.04]. There was no effect on BMI (P = 0.78), nor was there any significant genotype-BMI interaction on type 2 diabetes (P = 0.75).

Associations with body composition and metabolic characteristics: cross-sectional analyses. In the nondiabetic subgroup, subjects with Asp/Asp at position 1,057 had higher percent body fat (P = 0.01), BMI (P = 0.02), and waist circumference (P = 0.004), after adjustment for age and sex (Table 1). There was no difference in fasting or 2-h mixed meal test glucose and insulin concentrations, *M*-low, *M*-high, and average SAAS [0.77 ± 0.02 μg lipid/cell (X/Gly) and 0.78 ± 0.03 μg lipid/cell (Asp/Asp), adjusted for age, sex, waist circumference, and percent body fat; basal EGP and EGP during the clamp or AIR (additionally adjusted for *M*-low) all $P > 0.2$)] (Table 1). However, the slope of the curve for the associations between fasting glucose (P = 0.02), the AUC for glucose during the mixed meal test (P = 0.05), basal EGP (P = 0.06; Fig. 1), EGP during the clamp (P = 0.0007; Fig. 1), SAAS (P = 0.006), and AIR (P = 0.08, all P for interaction percent body fat*genotype; Fig. 2) with percent body fat was different between subjects with Asp/Asp compared with subjects with X/Gly. In a subgroup of 98 subjects, the association between plasma free fatty acids adjusted for age, sex, and percent body fat was marginally different between individuals with Asp/Asp and those with X/Gly (P = 0.07).

Associations with body composition and metabolic characteristics: longitudinal analyses. To examine whether the Gly1057Asp polymorphism has an effect on changes in anthropometrics and metabolic characteristics in people whose percent body fat changed, we analyzed longitudinal data for a group of 132 nondiabetic subjects with a mean follow-up time of 5.1 years. Change in metabolic parameters was investigated (follow-up adjusted for baseline) according to the genotype of the Gly1057Asp polymorphism in each tertile of change in percent body fat. In the upper tertile, subjects with Asp/Asp had a larger increase in BMI, percent body fat, and waist circumference than subjects with X/Gly. Asp/Asp homozygotes also had greater increases in fasting glucose and insulin concentrations, 2-h glucose and insulin concentrations, and the AUC for glucose and insulin concentrations during the mixed meal test and greater decreases in *M*-low and *M*-high (Table 2, Fig. 3). All these changes were statistically significant independent of age at follow-up, sex, and concomitant changes in percent body fat or waist circumference and time of follow up. Asp homozygote individuals in the upper tertile also had a greater increase in basal EGP (Fig. 1) and a greater decrease in AIR (Fig. 2) when additionally adjusted for change in *M*-low (Table 2). In the lowest tertile, subjects with X/Gly had a greater decrease in AIR (Fig. 2) and a greater increase in 2-h insulin (P = 0.03). None of the other changes in anthropometrics and metabolic characteristics

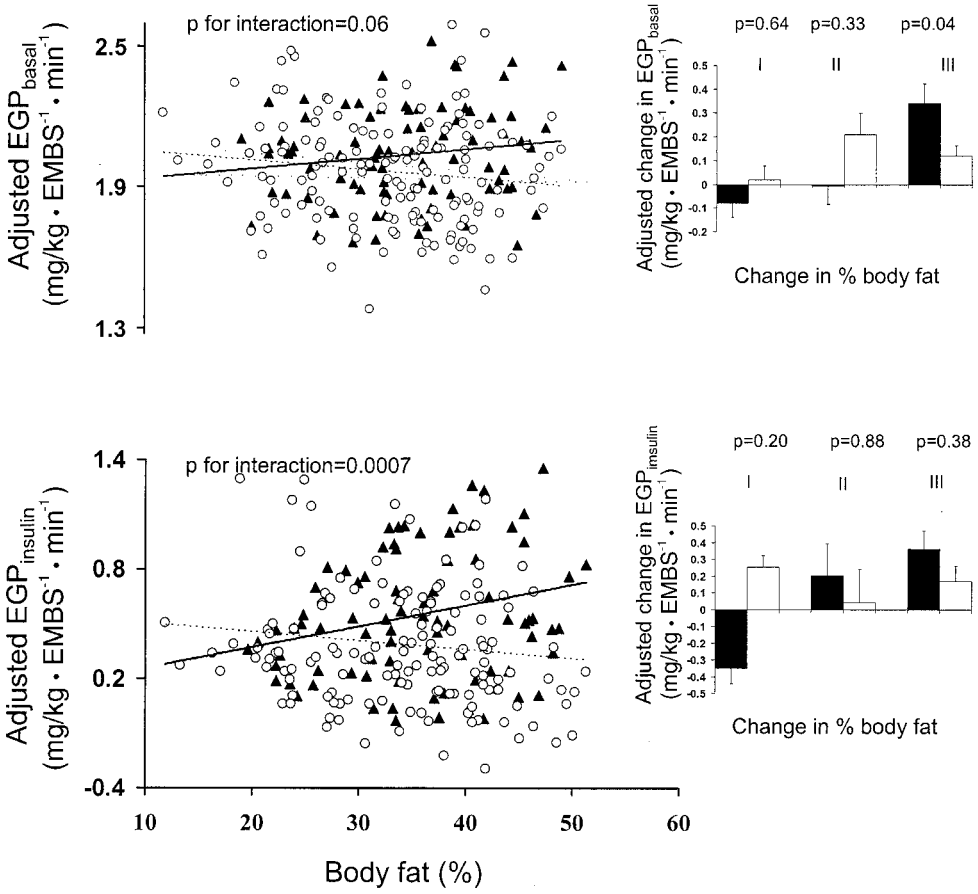

FIG. 1. Cross-sectional relationships between adjusted values of EGP in the basal state (EGP_{basal}) and during insulin infusion ($EGP_{insulin}$), with percent body fat for nondiabetic Pima Indians with X/Gly (○) and Asp/Asp (▲) according to the Gly1057Asp polymorphism in *IRS-2*. EGP was adjusted for age, sex, percent body fat, waist circumference, and *M*-low. The *P* values indicate statistical differences for interaction genotype*percent body fat. The inserts show the change ± SE in EGP in a subgroup of nondiabetic Pima Indians (■, Asp/Asp; □, X/Gly) who had measurements at the initial visit and at the follow-up visit, and who were divided in tertiles according to the change in percent body fat [change in percent body fat was −3.72 ± 0.46 (I), 1.29 ± 0.12 (II), and 6.77 ± 0.44 (III)]. The *P* values indicate statistical differences for change in EGP (values at the follow-up visit adjusted for values at the initial visit). Change in EGP was adjusted for age at the follow-up visit, time of follow-up, sex and percent body fat, waist circumference, and *M*-low at both the initial and follow-up visits.

between Asp/Asp and X/Gly in the lowest and in the middle tertiles were statistically significant ($P > 0.09$).

DISCUSSION

In Pima Indians, the frequency of the Asp1057 allele of the Gly1057Asp polymorphism in *IRS-2* is higher than in any other population reported to date (5–10). Compared with Caucasians, in whom the Gly1057 allele is the predominant one, the Asp1057 allele is the more frequent allele in Pima Indians. Subjects with Asp/Asp had a higher prevalence of type 2 diabetes. This finding was not dependent on whether subjects were also obese at the time of the diagnosis. However, in a subgroup of nondiabetic Pima Indians, the association of the polymorphism with fasting and postprandial glycemia, EGP, average SAAS, and AIR after an intravenous glucose challenge depended on whether subjects were lean or obese.

Having found an interaction of this polymorphism with obesity on metabolic characteristics in cross-sectional analyses, we found that among Pima Indians in the upper tertile of change in percent body fat, subjects with Asp/Asp had a greater decline in glucose tolerance, whole body insulin sensitivity, and AIR and a greater increase in basal EGP compared with Pima Indians with X/Gly. These differences were not fully explained by the greater increase in adiposity. Among subjects in the lowest and the middle tertiles of change in percent body fat, except for AIR and 2-h plasma insulin, there was no difference in change of anthropometrics and metabolic characteristics according to the genotype. This may be due to the fact that the magnitude of change in percent body fat was lower in the lowest and the middle tertiles. Therefore, we may not have had the power to detect more effects of the genotype on metabolic characteristics.

The newly discovered polymorphism at position 819, resulting in an amino acid substitution of aspartic acid to histidine, was not further investigated due to the low allelic frequency. We do not consider this variant to be an important determinant of metabolic characteristics in this population.

In a previous study in Italian Caucasians, a lower risk of diabetes in lean carriers of the Asp1057 allele and a higher risk in obese subjects (11) was reported. The protective effect of the Asp1057 allele in lean individuals was not confirmed in our study. One reason for this discrepancy includes that Pima Indians are more obese than the Caucasians in the Italian study. A different genetic background as clearly suggested by the difference in allelic frequency may also play a role.

We also investigated mechanisms through which this polymorphism may confer the increased risk of type 2 diabetes. In Pima Indians, obesity, low insulin sensitivity, high SAAS, and low insulin secretory response to a glucose challenge predict type 2 diabetes (20,27,28). Regarding obesity, the Asp/Asp genotype was associated with higher percent body fat, waist circumference, and BMI in the nondiabetic subgroup compared with the X/Gly genotype. In the larger cohort, however, in which data on percent body fat were not available, we did not find this association with BMI. We cannot explain this discrepancy at present.

Effects of the polymorphism on whole-body insulin

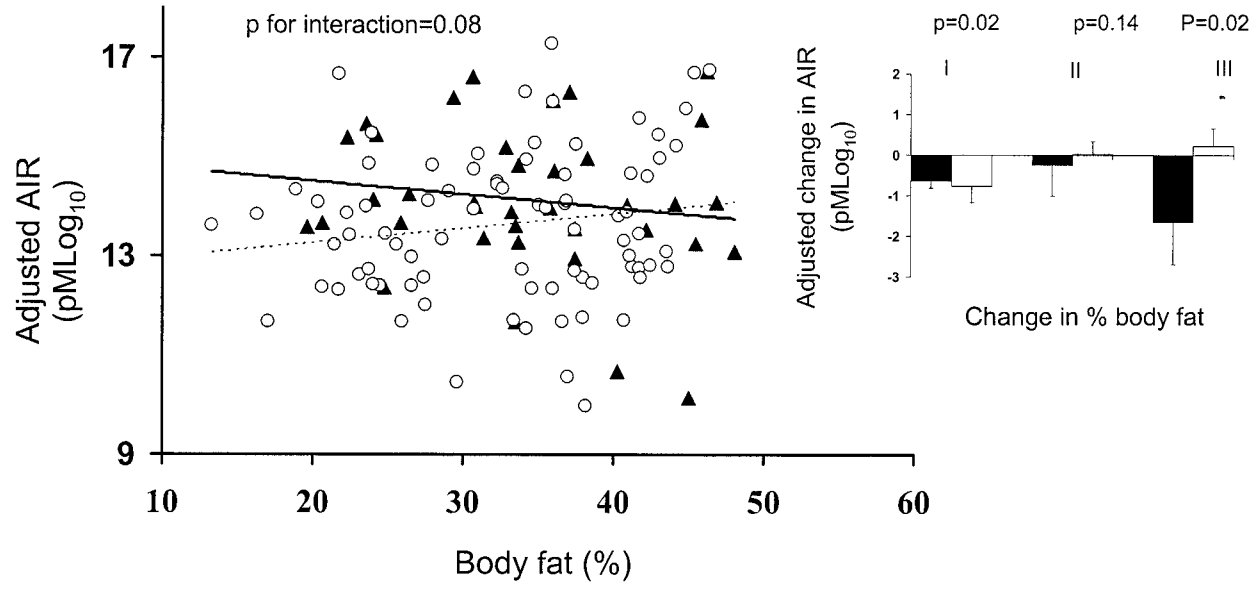

FIG. 2. Cross-sectional relationship between adjusted values of AIR with percent body fat for normal glucose-tolerant Pima Indians with X/Gly (○) and Asp/Asp (▲) according to the Gly1057Asp polymorphism in *IRS-2*. AIR was adjusted for age, sex, percent body fat, waist circumference, and insulin-stimulated glucose disposal during the low-dose insulin infusion. The *P* values indicate statistical differences for interaction genotype*percent body fat. The insert shows the change ± SE in AIR in a subgroup of normal glucose-tolerant Pima Indians (■, Asp/Asp; □, X/Gly) who had measurements at the initial visit and at the follow-up visit, and who were divided in tertiles according to the change in percent body fat [change in percent body fat was −3.72 ± 0.46 (I), 1.29 ± 0.12 (II), and 6.77 ± 0.44 (III)]. The *P* values indicate statistical differences for change in AIR (values at the follow-up visit adjusted for values at the initial visit). Change in AIR was adjusted for age at the follow-up visit, time of follow-up, sex and percent body fat, waist circumference, and *M*-low at both the initial and follow-up visits.

sensitivity were only seen in longitudinal analyses in subjects who had a relatively high increase in percent body fat. The fact that there was no interaction of the genotype with obesity on whole-body insulin sensitivity in cross-sectional analyses suggests that these differences may not be large enough to be detected in these types of analyses. However, the cross-sectional relationships of percent body fat with glycemia, basal EGP, and suppression of EGP during the clamp were significantly different in the Asp/Asp group compared with the X/Gly group. Most of these associations were confirmed in the longitudinal analyses. Suppression of EGP reflects hepatic insulin sensitivity. Possible mechanisms by which this polymorphism can affect hepatic insulin sensitivity include primary impairment of hepatic insulin signaling and effects secondary to increased availability of plasma free fatty acid concentrations. Data on the effects of the polymorphism on hepatic insulin signaling are not available. Probably of

TABLE 2
Anthropometrics and metabolic characteristics of Pima Indians who were in the upper tertile (*n* for all subjects = 132) of change in percent body fat in longitudinal analyses and who were genotyped for Gly1057 Asp in *IRS-2*

	X/Gly [*n* = 33 (20M/13F)]		Asp/Asp [*n* = 11 (6M/5F)]		
	Initial	Follow-up	Initial	Follow-up	*P**
Age (years)	24 ± 1	31 ± 1	25 ± 2	31 ± 2	—
BMI (kg/m^2)	31 ± 1	36 ± 1	31 ± 2	38 ± 3	0.02
Body fat (%)	28 ± 1	35 ± 1	31 ± 2	38 ± 2	0.006
Waist circumference (cm)	102 ± 3	112 ± 3	102 ± 5	117 ± 8	0.06
Fasting glucose (mmol/l)	4.95 ± 0.08	5.10 ± 0.08	4.84 ± 0.08	5.33 ± 0.19	0.0001
2-h glucose (mmol/l)	6.77 ± 0.23	7.81 ± 0.27	6.71 ± 0.54	7.87 ± 0.77	0.007
Fasting insulin (pmol/l)	207 ± 22	300 ± 32	198 ± 38	312 ± 60	0.0001
2-h insulin (pmol/l)	1212 ± 162	1584 ± 174	1158 ± 456	2172 ± 828	0.0001
Glucose $AUC_{meal\ test}$ (mmol/l · 240 min)	1324 ± 15	1406 ± 27	1319 ± 22	1522 ± 88	0.002
Insulin $AUC_{meal\ test}$ (pmol/l · 240 min)	148,908 ± 15,786	189,924 ± 19,626	134,862 ± 20,688	265,854 ± 82,446	0.03
EGP_{basal} (mg/kg · $EMBS^{-1}$ · min^{-1})	1.90 ± 0.04	2.04 ± 0.04	1.95 ± 0.08	2.20 ± 0.07	0.04
$EGP_{insulin}$ (mg/kg · $EMBS^{-1}$ · min^{-1})	0.35 ± 0.06	0.55 ± 0.06	0.39 ± 0.14	0.69 ± 0.12	0.38
M-low (mg/kg · $EMBS^{-1}$ · min^{-1})	2.64 ± 0.14	2.33 ± 0.14	2.83 ± 0.31	2.26 ± 0.20	0.03
M-high (mg/kg · $EMBS^{-1}$ · min^{-1})	8.76 ± 0.37	7.95 ± 0.36	9.04 ± 7.28	7.28 ± 0.68	0.0001
AIR (pmol/l)†	1,800 ± 396	1,734 ± 240	1,578 ± 312	1,500 ± 342	0.02

Values are mean ± SE. **P* for statistical differences in change between the groups; †24 subjects who were normal glucose tolerant at the initial visit. Change (follow-up value adjusted for initial value) in anthropometrics was adjusted for age at the follow-up visit and sex. Changes in plasma glucose, insulin concentrations, and *M* values were adjusted for age at the follow-up visit, sex, change in waist circumference, and change in percent body fat. Changes in EGP and AIR were additionally adjusted for change in *M*-low. EMBS = fat-free mass + 17.7 kg. Time of follow-up = 6.4 ± 1 years.

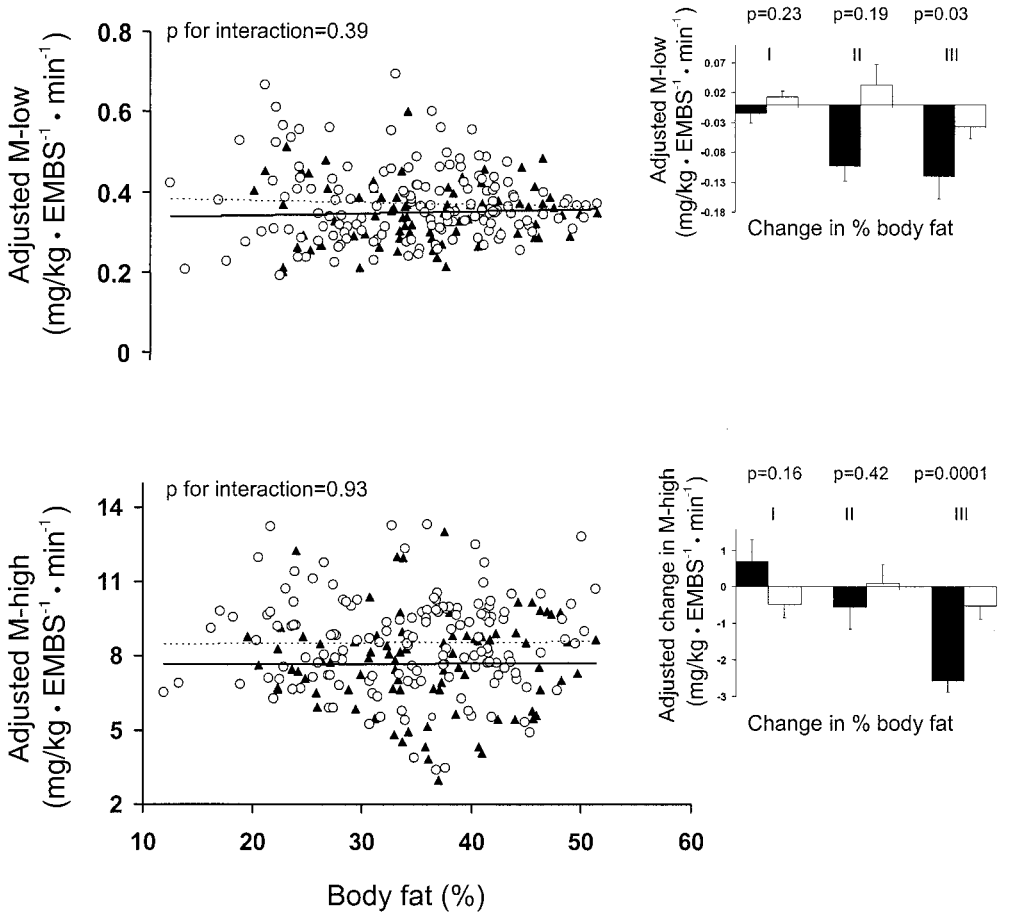

FIG. 3. Cross-sectional relationships between adjusted values of insulin-stimulated glucose disposal during high-dose (M-high) and low-dose (M-low) insulin infusion with percent body fat for nondiabetic Pima Indians with X/Gly (○) and Asp/Asp (▲) according to the Gly1057Asp polymorphism in *IRS-2*. *M* was adjusted for age, sex, percent body fat, and waist circumference. The *P* values indicate statistical differences for interaction genotype*percent body fat. The inserts show the change ± SE in *M* in a subgroup of nondiabetic Pima Indians (■, Asp/Asp; □, X/Gly) who had measurements at the initial visit and at the follow-up visit, and who were divided in tertiles according to the change in percent body fat [change in percent body fat was −3.72 ± 0.46 (I), 1.29 ± 0.12 (II), and 6.77 ± 0.44 (III)]. The *P* values indicate statistical differences for change in *M* (values at the follow-up visit adjusted for values at the initial visit). Change in *M* was adjusted for age at the follow-up visit, time of follow-up, sex and percent body fat, and waist circumference at both the initial and follow-up visits.

less relevance, but nevertheless noteworthy, we found an interaction of the polymorphism with obesity on plasma free fatty acid concentrations in a small group of Pima Indians. Enlarged fat cells from subcutaneous adipose tissue have been shown to have increased lipolytic activity (29), resulting in elevated plasma free fatty acid levels. Consistent with this, Pima Indians homozygous for the Asp1057 allele had higher average SAAS, in parallel with elevated plasma free fatty acids, when they were obese. *IRS-2* was shown to play an essential role in differentiation of preadipocytes into adipocytes (30). Thus, it is possible that this polymorphism in *IRS-2* modulates triglyceride accumulation and, hence, cell size in a specific milieu, such as that of caloric oversupply and obesity.

IRS-2 was also shown to play an important role in insulin secretion. *IRS-2* knockout mice had an impaired glucose-stimulated insulin secretion (2). Furthermore, *IRS-2* signaling was shown to be important for development of β-cells (31) and regulation of β-cell mass (4). We found a different association of the polymorphism with AIR during an intravenous glucose tolerance test in Pima Indians, which depended on whether subjects were lean or obese. Therefore, only in obese subjects we found a human correlate to the animal knockout mouse model with respect to β-cell function.

Comparable results were found in 212 normal glucose-tolerant Caucasians from the German Tübingen Family Study for type 2 diabetes. In this population the association of the Gly1057Asp polymorphism with 30-min C-peptide plasma concentrations during an OGTT (surrogate measure of glucose-stimulated insulin secretion) depended on whether subjects were lean or obese (personal communication, M.S.). Taken together, these data are suggestive of a deleterious effect of this polymorphism on insulin secretory function under conditions of increased demand such as obesity.

As there is no information on the effect of this polymorphism on the molecular function of *IRS-2*, explanations for the observed interaction with obesity remain speculative. Aspartic acid in contrast to glycine is a charged amino acid. The exchange is located close to two putative tyrosine phosphorylation sites (at positions 1,042 and 1,072) of the protein. Therefore, alterations in downstream signaling through *IRS-2* may be involved. It remains to be determined which obesity-related factors interfere with *IRS-2* signaling to make any functional significance of the polymorphism become apparent. However, there is the possibility that this polymorphism is not functional but may be in linkage disequilibrium with a currently unrecognized functional polymorphism.

In conclusion, our findings suggest that the association of homozygosity for the Asp1057 allele in *IRS-2* with type 2 diabetes in Pima Indians may be mediated by interaction of the polymorphism with obesity on several diabetes-related traits. This polymorphism, therefore, may serve as an important genetic variant to study gene-environment interaction on type 2 diabetes.

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