The Gly1057Asp polymorphism in *IRS-2* interacts with obesity to affect beta cell function

To the editor: Insulin receptor substrate (IRS)-2 plays an important role in insulin signalling and its disruption, in mice, results in diabetes [1]. This could be attributed largely to hepatic insulin resistance and lack of beta cell compensation [2]. Although *IRS-2* knock-out mice have a similar body weight to wild-type mice, they have twice as much body fat [3]. Because the severity of diabetes increases rapidly with weight gain in these mice, it can be hypothesised that a lack of *IRS-2* is especially important in the development of obesity.

In humans, a number of polymorphisms have been identified in the *IRS-2* gene, the most common of which is represented by the Gly1057Asp substitution. We have previously investigated whether this polymorphism is associated with pre-diabetic phenotypes in non-diabetic people in our population from southern Germany. We did not find associations between the polymorphism and insulin sensitivity or insulin secretory function [4]. In an Italian population, the Gly1057Asp substitution was associated with a lower risk of Type 2 diabetes in lean people, but with a higher risk in obese people [5]. We therefore tested whether this polymorphism interacts with adiposity to affect insulin sensitivity and insulin secretory function in our population.

In our analysis we included 202 subjects with normal glucose tolerance [WHO criteria [6], (OGTT group)] from our Tübingen Family Study database. Subjects were studied using an OGTT and a euglycaemic hyperinsulinaemic clamp. A subgroup of 69 people (clamp group) underwent a modified hyperglycaemic clamp [7], and a third group of 20 people (lipid group) underwent two hyperglycaemic clamps (8 mmol/l, 140 min) before and after 5 h of infusion of Intralipid and heparin. For this study we obtained the approval of the local ethics committee and the consent of the families involved.

The C-peptide plasma concentrations at 30 min during the OGTT were used as an estimate of beta cell function in the OGTT group. The first and second phase of C-peptide secretion during the hyperglycaemic clamp was calculated as the sum of the C-peptide concentrations from 2.5 to 10 min, and from 80 to 120 min respectively in the clamp group and the lipid group. Insulin sensitivity according to the insulin sensitivity index was measured during the hyperglycaemic clamp by relating the glucose infusion rate to the plasma insulin concentration during the second hour [7]. For statistical comparisons, an additive model and a recessive model were used [homozygotes (Asp/Asp) for the rare allele vs heterozygotes (Gly/Asp) and homozygotes (Gly/Gly) for the frequent allele]. The secretion indices of the OGTT and the clamp were log transformed and linearly adjusted for insulin sensitivity, percentage of body fat, waist-to-hip ratio, age and sex. The variables of primary interest were genotype and the interaction term of genotype x percentage body fat.

In the OGTT group and the clamp group the genotype did not interact with the percentage of body fat to affect fasting and post-prandial glucose concentrations or insulin sensitivity ($p>0.17$ for all). However, in the OGTT group the slope of the curve for the associations between plasma C-peptide concentrations at 30 min and percentage of body fat (Fig. 1a) was different depending on the genotype (additive model $p=0.004$, recessive model $p=0.03$, adjusted for covariates). This statistical significance was mostly due to the homozygous Asp/Asp carriers. Similar results were seen in the clamp group for the associations between C-peptide secretion and percentage of body fat for the first phase (additive model $p=0.005$, recessive model $p=0.04$) and the second phase (additive model $p=0.003$, recessive model $p=0.09$). During lipid infusion there was an increase of C-peptide secretion in the first phase in subjects with Gly/Gly (104±219 pmol/l), and a decrease in subjects carrying the Asp1057 allele [–477±154 pmol/l (Gly/Asp) and −543±219 pmol/l (Asp/Asp), means ± SE after adjustment for BMI and change in insulin sensitivity, ANOVA $p=0.07$]. Similar results, although not statistically significant, were seen for the second phase [+37±54 pmol/l (Gly/Gly), −57±38 pmol/l (Gly/Asp) and −68±54 pmol/l (Asp/Asp), ANOVA $p=0.14$, Fig. 1b].

In our analysis we found that the Gly1057Asp polymorphism in *IRS-2* interacts with obesity to affect beta cell function. The association of the polymorphism with C-peptide plasma concentrations at 30 min during an OGTT (surrogate measure of glucose-stimulated insulin secretion), and with the first and second phase of C-peptide secretion during the hyperglycaemic clamp, depended on whether the subjects were lean or obese. In a small group with normal glucose tolerance who underwent a lipid infusion protocol an increase in plasma non-esterified fatty acids impaired insulin secretion in carriers of the Asp1057 allele, while there was a small increase in subjects who were homozygous for the Gly1057 allele. These data suggest that this polymorphism has a deleterious effect on beta cell function under conditions of increased demand, such as obesity or increased concentrations of plasma non-esterified fatty acids. Our results are consistent with similar findings in Pima Indians. In non-diabetic Pima Indians the associations between this polymorphism and several metabolic characteristics depended on whether people were lean or obese [8]. The relationship between body fat and insulin-stimulated glucose disposal, endogenous glucose production and acute insulin response was significantly different in the group homozygous for the Asp1057 allele when compared with the group heterozygous and/or homozygous for the Gly1057 allele. We did not find the polymorphism to interact with obesity and affect insulin sensitivity.

In conclusion, our findings suggest that, in our population, the Gly1057Asp polymorphism in *IRS-2* interacts with obesity to affect beta cell function. These findings support the concept

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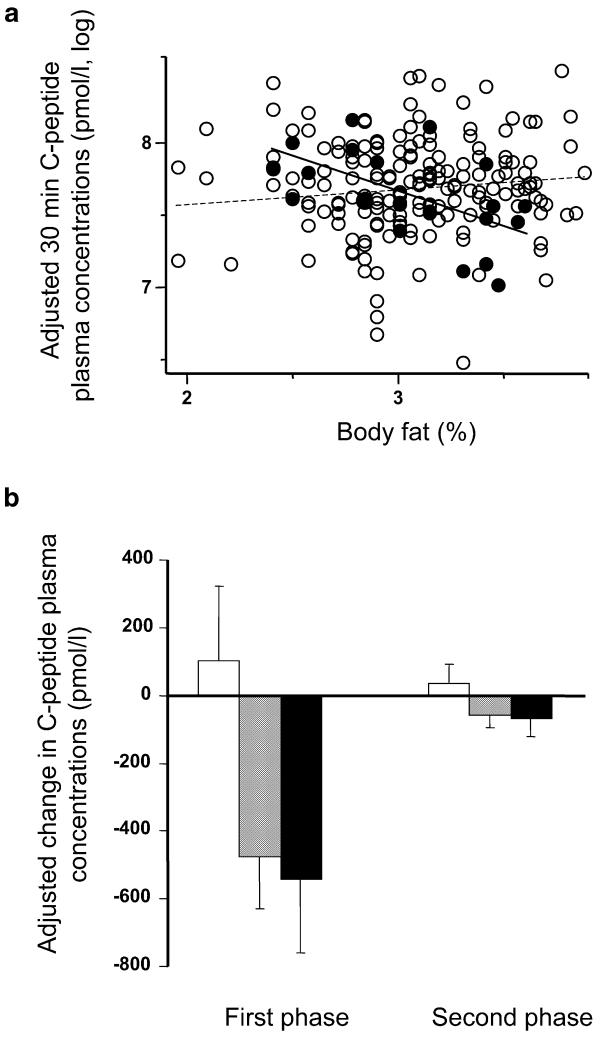

Fig. 1. (**a**) Relationship between C-peptide plasma concentrations at 30 min during the OGTT (adjusted for age, sex, waist-to-hip ratio, percentage of body fat and insulin sensitivity) and percentage of body fat for people with normal glucose tolerance. Depending on the Gly1057Asp polymorphism in *IRS-2*, white circles represent X/Gly and black circles Asp/Asp. p=0.03 for the genotype x percentage body fat interaction. (**b**) Change in first and second phase of C-peptide secretion (2^{nd} minus 1^{st} hyperglycaemic clamp) after lipid infusion for people with Gly/Gly (white bars), Gly/Asp (grey bars) and Asp/Asp (black bars) according to the Gly1057Asp polymorphism in *IRS-2*. Values were adjusted for BMI and change in insulin sensitivity. ANOVA p value for statistical difference between the genotype groups: first phase p=0.07, second phase p=0.14

that this polymorphism serves as an important genetic variant in the study of gene x environment interaction and affects beta cell function.

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