

Clinical Characterization of Insulin Secretion as the Basis for Genetic Analyses

Michael Stumvoll, Andreas Fritsche, and Hans-Ulrich Häring

A strong genetic component of the β -cell defect of type 2 diabetes is undisputed. We recently developed a modification of the classic hyperglycemic clamp to assess β -cell function in response to various stimuli (10 mmol/l glucose, additional glucagon-like peptide [GLP]-1, and arginine). Subjects at risk for developing type 2 diabetes (impaired glucose-tolerant individuals, women with gestational diabetes, and individuals with a family history of type 2 diabetes) clearly showed a significantly decreased mean secretory response to all secretagogues compared with controls. We also showed that normal glucose-tolerant carriers of the Gly972Arg polymorphism in the insulin receptor substrate 1 have significantly reduced insulin secretion in response to glucose and arginine but not to GLP-1. More remarkably, however, the relative impairment of the different secretory phases varied greatly in the same individual, indicating a substantial heterogeneity of β -cell dysfunction. Specific prominence of this heterogeneity may reflect a specific cellular defect of the β -cell. In subjects sharing this pattern of heterogeneity, any underlying genetic variant may be enriched and thus more likely not only to be identified but also to be related to a pathophysiological mechanism. In conclusion, we believe that careful clinical characterization of β -cell function (and dysfunction) is one way of identifying and understanding the genetic factors leading to the insulin secretory failure of type 2 diabetes. *Diabetes* 51 (Suppl. 1): S122-S129, 2002

Type 2 diabetes is a heterogeneous disorder characterized by varying degrees of impaired insulin secretion and insulin resistance. Although the site of the primary genetic lesion (β -cell or insulin target tissues) is still a matter of controversy, it is generally agreed that 1) the disease has strong genetic and environmental (acquired) components (1–4); 2) its mode of inheritance is polygenic (5–7), meaning that several genetic abnormalities are required for disease manifestation; and 3) the variation in β -cell function segregates among family members (4,8,9), indicating that β -cell dys-

function has a relevant genetic component. On the other hand, what is still unclear is the relative impact of genetic versus environmental factors and the precise nature of the genetic lesion(s) responsible for β -cell dysfunction in common type 2 diabetes.

It is worth noting that entirely different molecular defects (for example, in the “glucose sensor” glucokinase and the transcription factor hepatocyte nuclear factor 1 α) can result in the clinical phenotype of abnormal insulin secretion (10). This result strongly indicates that abnormalities in many different genes may be involved in β -cell dysfunction. In addition, different polymorphisms in the same gene can have different functional consequences, as illustrated by mutations in the insulin promoting factor (IPF)-1. A homozygote frameshift mutation resulted in agenesis of the pancreas and in maturity-onset diabetes of the young in heterozygotes (11), whereas several missense mutations led only to a late-onset type 2 diabetic phenotype (12–14). It is possible that less detrimental, still unidentified genetic variants of IPF-1 contribute to impaired insulin secretion to a minor degree, which may be difficult to assess in vivo. The impairment in β -cell function by such a mutation alone may not be sufficient to result in overt hyperglycemia. However, in combination with other minor (or major) genetic factors, it may well be the straw that breaks the camel’s back.

Among the experimental strategies used to identify genetic factors of β -cell failure, the candidate gene approach has not been very successful, with the exception of rare monogenetic forms of type 2 diabetes (e.g., detrimental point mutations in the glucokinase gene). On the other hand, positional cloning based on linkage analysis of many pooled pedigrees led to the identification of a mutation in calpain 10 (15), which is now considered a true diabetes gene. However, the functional relevance of this cysteine protease and its connection with glucose metabolism is still unclear (16). There is evidence for a functional involvement of calpain 10 in muscle and β -cells. Moreover, studies both in knockout animals (17) and transfected β -cells (18) have produced intriguing results regarding the role of abnormal signaling through the insulin receptor substrate (IRS) molecules in insulin secretion, which still await translation into the clinical context. This obvious complexity suggests that a more careful clinical characterization of β -cell function may be required as the basis for state-of-the-art genetic analyses.

However, the characterization of potential subphenotypes of type 2 diabetes after the onset of overt hyperglycemia and other metabolic derangements is virtually impossible because hyperglycemia per se impairs both insulin sensitivity and secretion, masking any primary

From the Medizinische Klinik, Abteilung für Endokrinologie, Stoffwechsel und Pathobiochemie, Eberhard-Karls-Universität, Tübingen, Germany.

Address correspondence and reprint requests to michael.stumvoll@med.uni-tuebingen.de.

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ANOVA, analysis of variance; AUC, area under the curve; GDM, gestational diabetes mellitus; GLP, glucagon-like peptide; IGT, impaired glucose tolerance; IPF, insulin promoting factor; IRS, insulin receptor substrate; OGTT, oral glucose tolerance test.

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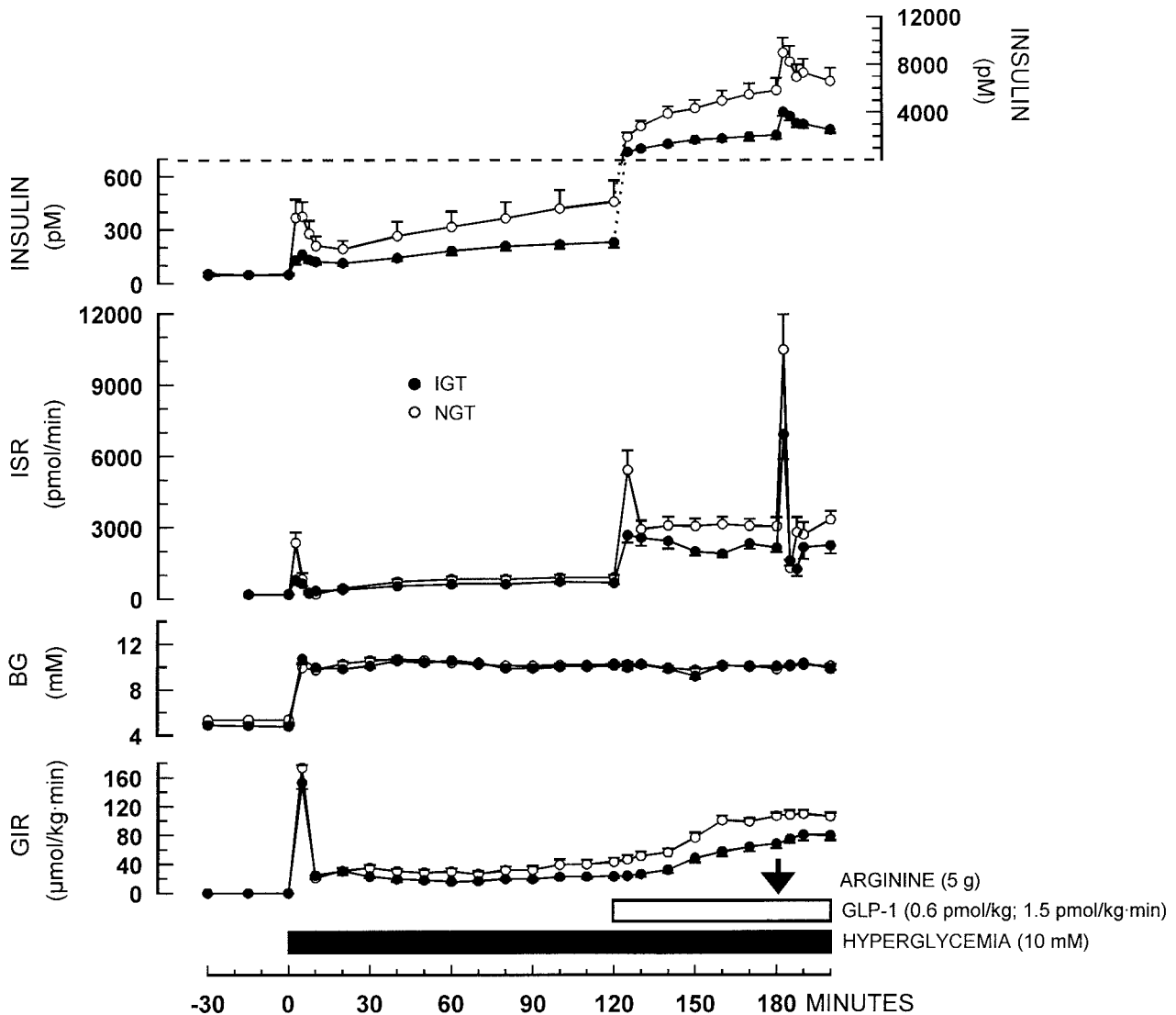


FIG. 1. Glucose infusion rates (GIR), blood glucose concentration (BG), insulin secretion rates (ISR) (calculated from plasma C-peptide concentrations by deconvolution), and plasma concentrations of insulin at baseline and during 200 min of the hyperglycemic clamp in normal glucose-tolerant (NGT) and IGT subjects. Note that for reasons of clarity, the *y*-axis for insulin was split and continued on the right with a different scale (35).

abnormality. It is thus necessary to study cohorts at an increased genetic risk of developing type 2 diabetes before the onset of overt hyperglycemia. These cohorts include subjects with impaired glucose tolerance, normal glucose-tolerant individuals with a family history of type 2 diabetes, and women with a history of gestational diabetes. In Tübingen (southwestern Germany), we clinically characterize such populations by a variety of clinical-experimental methods as the basis for genetic association studies (euglycemic clamp for insulin sensitivity, stable isotopes for lipolysis and glucose production, magnetic resonance spectroscopy for intramyocellular lipids, flow-associated vasodilation for endothelial dysfunction, and hyperglycemic clamp for β -cell function). In addition to classifying insulin secretion (high vs. low) by a standard hyperglycemic clamp, we made an attempt to quantify different aspects of insulin secretion by measuring the secretory response to different secretagogues in the same subjects.

The hypothesis is that this might result in patterns of abnormal β -cell function specific for a certain genotype.

We therefore designed a hyperglycemic clamp (10 mmol/l) in which the classic 2-h square-wave hyperglycemia is followed by additional administration of glucagon-like peptide 1 (GLP-1) ($1.5 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ over 80 min) and a final arginine bolus (5 g) (19). The test produced a distinct pattern of plasma insulin and C-peptide concentrations in response to the three secretagogues. First and second phases of glucose-stimulated insulin secretion, an early and a late phase of GLP-1-induced insulin secretion, and an acute response to arginine could be calculated. Moreover, the combination of secretagogues used in this clamp resulted in insulin and C-peptide concentrations that exceeded those seen in the test hitherto considered to provoke the maximal insulin response by more than 100% (arginine at $>25 \text{ mmol/l}$ glucose [20]). The maximal serum insulin and C-peptide concentrations observed during this

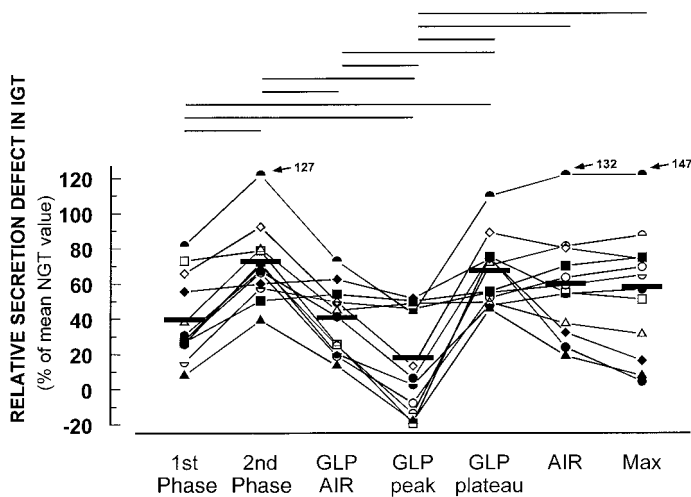


FIG. 2. Relative impairment of insulin secretion in response to different secretagogues in subjects with IGT. Data are expressed as percent of insulin secretion in subjects with normal glucose tolerance (NGT). Horizontal lines denote statistical difference ($P < 0.05$) using Duncan's procedure for multiple comparisons (35). Negative values for the peak response to GLP-1 result from expressing the peak as the value above the subsequent plateau. AIR, acute insulin response.

test may come closest to the assessment of the maximal insulin secretory capacity in humans in vivo. The novel test takes 3.5 h and was shown to be highly reproducible (19). It can be used in various contexts requiring differential characterization of islet function in selected human populations. In addition, insulin sensitivity can be determined by relating the glucose infusion rate during the second phase of the hyperglycemic clamp to the insulin concentration during the same interval.

STUDIES IN PEOPLE WITH IMPAIRED GLUCOSE TOLERANCE

Our first application of this test was its use in a group of subjects with impaired glucose tolerance (IGT) compared with a normal glucose-tolerant control group that was perfectly matched for BMI, waist-to-hip ratio, sex, and age (21). Most phases of insulin secretion were significantly reduced in the subjects with IGT (Fig. 1). Insulin sensitivity was not significantly different between the two groups. This was a result of the matching process, which excluded the impact of obesity, body fat distribution, age, and sex. The novel finding, however, was that the group with IGT subjects did not behave homogeneously with respect to the relative impairment of the different phases. Displaying the individual data makes it apparent that the pattern of impairment varies greatly among subjects (Fig. 2). For example, one subject (Fig. 2, filled diamonds) reached 55% of the normal first phase but not even 20% of the average maximal secretion. Another subject (Fig. 2, open semicircle, upper half) produced an almost normal maximal secretion, whereas the GLP peak was entirely absent. A particularly surprising finding of the study was the absence of the sharp peak in insulin secretion rate in response to the start of the GLP infusion (after deconvolution of C-peptide concentrations) in the group with IGT. This may indicate that, at least in a subgroup, a β -cell defect specifically in the GLP-1-dependent signaling pathway is involved in the pathogenesis of β -cell failure. In

conclusion, it is very obvious that the impairment of insulin secretion varies greatly among subjects with IGT not only quantitatively but also qualitatively. A precise understanding of the individual abnormality may be essential for the identification of the genetic lesion(s) involved.

STUDIES IN NORMAL GLUCOSE-TOLERANT SUBJECTS WITH A FAMILY HISTORY OF TYPE 2 DIABETES

A total of 50 and 25% of genes are shared among first- and second-degree relatives, respectively. Twin studies showed concordance rates for any abnormality of glucose metabolism (either type 2 diabetes or impaired glucose tolerance) as high as 96% (22), underlining the substantial contribution of genetic factors. Furthermore, 40% of first-degree relatives of patients with type 2 diabetes develop the disease as opposed to $\sim 6\%$ of the general population (23). Moreover, there is evidence from different laboratories demonstrating subtle but significant abnormalities in β -cell function in first-degree relatives (4,9,24). We applied our modified hyperglycemic clamp to subjects with a family history of type 2 diabetes to determine specific patterns of β -cell response to different secretagogues.

All phases of insulin secretion were reduced to various extents in the subjects with a diabetic relative ($n = 37$) compared with the group without a known family history of type 2 diabetes ($n = 20$) (Fig. 3). Similar to the analysis of the group with IGT, first-phase ($2,243 \pm 168$ vs. $3,188 \pm 361$ pmol/min, $P = 0.02$) and GLP-1-induced peak insulin secretion rates ($1,561 \pm 175$ vs. $2,390 \pm 480$ pmol/min, $P = 0.02$) were significantly lower, whereas second phases and the response to arginine were not significantly different ($P > 0.20$ for both). A similar heterogeneity of β -cell dysfunction to that in the group with IGT was detectable in this normal glucose-tolerant group with an increased genetic risk for type 2 diabetes.

STUDIES IN GLUCOSE-TOLERANT WOMEN WITH A HISTORY OF GESTATIONAL DIABETES

Women with transient diabetes during pregnancy (gestational diabetes mellitus [GDM]) carry an increased risk of developing type 2 diabetes later in life (25). It has been suggested that GDM and type 2 diabetes are the same disorder (26). Therefore, it is likely that both have similar underlying genetic abnormalities. GDM occurs when the physiological reduction in insulin sensitivity during the second half of pregnancy cannot be countered by an adequate increase in insulin secretion. Thus, GDM represents a natural fast motion model of conditions such as obesity where a (possibly genetic) inability of the β -cell to compensate for reduced insulin sensitivity over the years results in type 2 diabetes.

Again, all phases of insulin secretion were more or less reduced in women with GDM ($n = 7$) compared with women with no evidence of glucosuria or a large-for-date baby ($n = 20$) (Fig. 4). First-phase insulin secretion ($1,769 \pm 239$ vs. $2,317 \pm 233$ pmol/min, $P = 0.04$) was significantly lower in women with GDM, whereas the second phase, the GLP-1-induced peak, and the response to arginine were not significantly different ($P > 0.15$ for all). Also in this group of women with GDM, a marked intra-individual heterogeneity in the response to the different secretagogues was observed, indicating that a num-

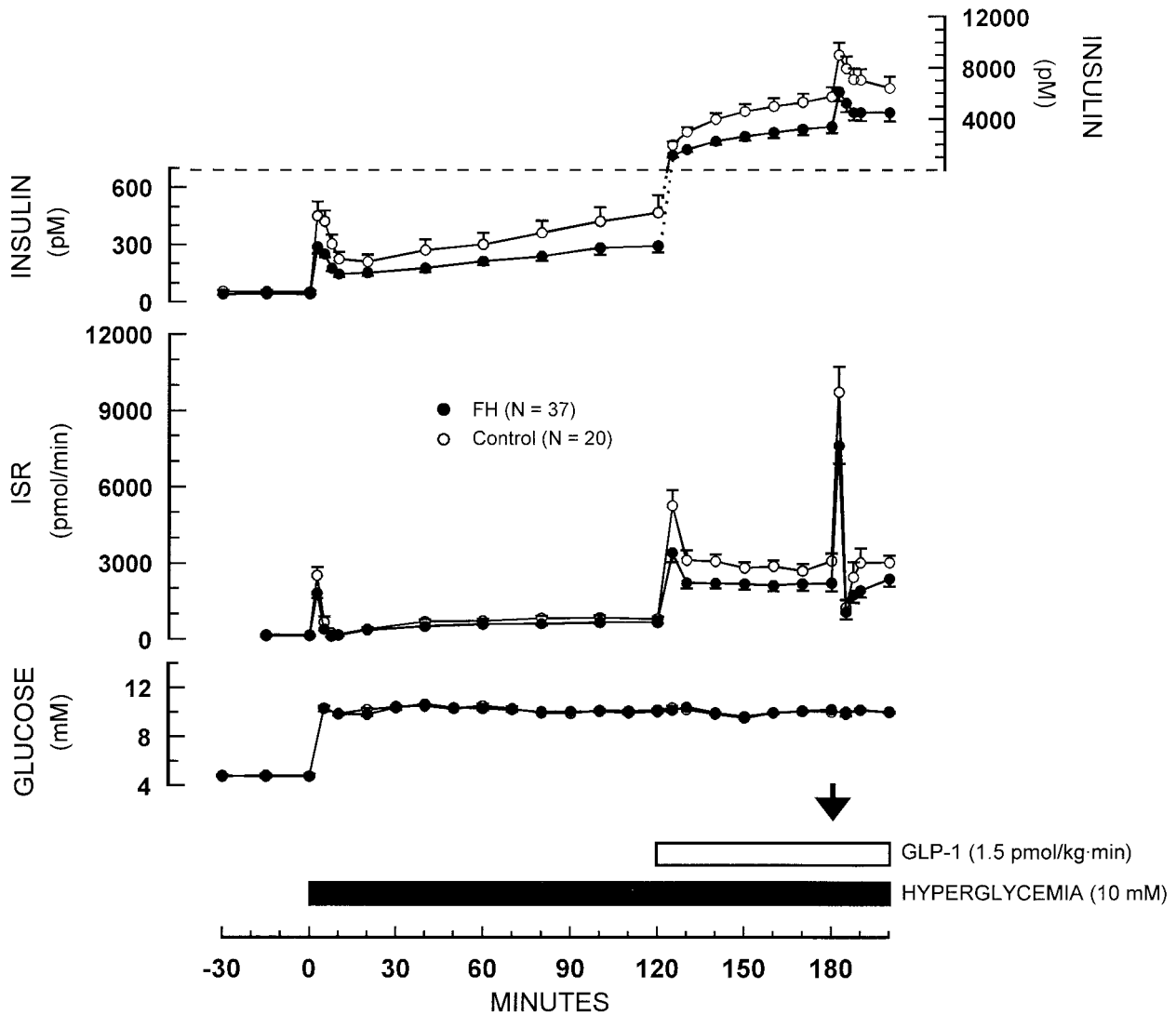


FIG. 3. Blood glucose concentrations, insulin secretion rates (ISR) (calculated from plasma C-peptide concentrations by deconvolution), and plasma concentrations of insulin at baseline and during 200 min of the hyperglycemic clamp in normal glucose-tolerant subjects with and without a family history (FH) of type 2 diabetes. Note that for reasons of clarity, the *y*-axis for insulin was split and continued on the right with a different scale.

ber of different (possibly genetically determined) factors modulate the response to the different secretagogues.

STUDIES IN CARRIERS OF THE GLY972ARG POLYMORPHISM IN IRS-1

In addition to analyzing insulin secretion in populations with a well-established reduction in insulin secretion, we assessed whether a specific genetic variant contributes to the interindividual variation of β -cell function. The Gly972Arg polymorphism in IRS-1 originally represented a prime candidate for genetic causes of impaired insulin signaling. Over the years, however, conflicting data accumulated (27–33), and this polymorphism has not held up to the expectations as an insulin resistance gene. Recently, it was shown that insulin-secreting cells overexpressing the IRS-1 Gly972Arg variant had a decreased sulfonylurea- and glucose-stimulated insulin secretion compared with cells overexpressing the wild-type IRS-1 (18). Moreover, cell apoptosis of isolated human islets obtained from organ donors heterozygous for Arg972 was increased twofold

compared with wild-type IRS-1 carriers. It was consequently suggested that this polymorphism might represent a genetic variant unifying insulin resistance and β -cell dysfunction. We studied 8 subjects with and 36 subjects without the polymorphism using the modified hyperglycemic clamp method. To provide greater statistical power, we also analyzed oral glucose tolerance tests (OGTTs) of 212 subjects (31 with and 181 without the mutation) using a number of recently validated indexes to estimate β -cell function from insulin (and C-peptide) concentrations obtained during an OGTT.

During the modified hyperglycemic clamp, insulin secretion rates were significantly lower in Gly/Arg compared with Gly/Gly during the first phase ($1,711 \pm 142$ vs. $3,014 \pm 328$ pmol/min, $P = 0.05$) and after maximal stimulation with arginine ($5,340 \pm 639$ vs. $9,075 \pm 722$ pmol/min, $P = 0.03$). During second-phase insulin secretion, the differences approached statistical significance, whereas during the GLP-1 phases, they were not significant (Fig. 5). Several validated indexes of β -cell function from the OGTT

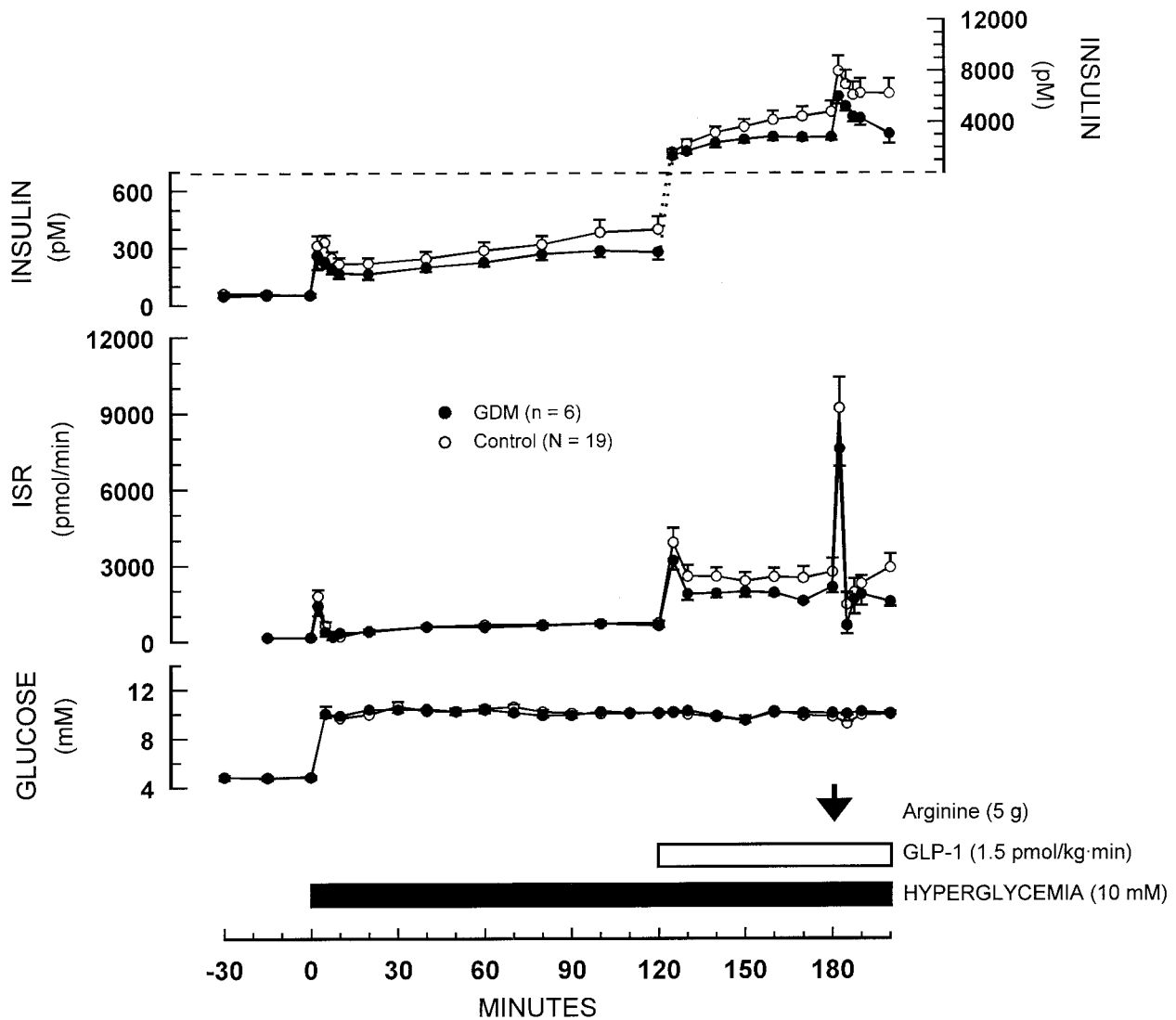


FIG. 4. Blood glucose concentrations, insulin secretion rates (ISR) (calculated from plasma C-peptide concentrations by deconvolution), and plasma concentrations of insulin at baseline and during 200 min of the hyperglycemic clamp in normal control women and women with GDM. Note that for reasons of clarity, the *y*-axis for insulin was split and continued on the right with a different scale.

were significantly lower in X/Arg ($n = 31$) compared with Gly/Gly ($n = 181$) (P between 0.002 and 0.05). Insulin sensitivity, as determined by the euglycemic-hyperinsulinemic clamp, was not different. In a subanalysis, we compared subjects with a BMI greater or less than 25 kg/m^2 . The C-peptide area under the curve (AUC)/glucose $_{\text{AUC}}$ ($\text{CP}_{\text{AUC}}/\text{G}_{\text{AUC}}$) was significantly greater in the Gly/Gly obese ($n = 69$) than in the Gly/Gly lean subjects ($n = 112$) (461 ± 17 vs. 373 ± 10 , $P < 0.001$). In contrast, the $\text{CP}_{\text{AUC}}/\text{G}_{\text{AUC}}$ was virtually identical between the lean ($n = 19$) and obese ($n = 12$) subjects in the X/Arg group (336 ± 25 vs. 341 ± 27 , $P = 0.91$; $P = 0.078$, analysis of variance [ANOVA]) (Fig. 6). In summary, our results suggest that the Gly972Arg polymorphism in IRS-1 is associated with decreased insulin secretion in response to glucose. It is possible that this polymorphism contributes to the variation in insulin secretion in normal glucose-tolerant humans. Although with ANOVA the interaction effect showed only a tendency, it is possible that this contribution may be particularly important in the presence of obesity.

HETEROGENEITY OF B-CELL FUNCTION

In addition to simply quantifying the insulin secretory response to glucose, the modified hyperglycemic clamp permits the assessment of the differential secretory response to different secretagogues, i.e., the “heterogeneity of insulin secretion.” To quantify this heterogeneity, we performed the following procedures: in a first step, the different phases of insulin secretion (first phase, second phase, peak after GLP-1, peak after arginine) are normalized by the population mean of each phase to account for the huge absolute differences (e.g., between second phase and the peak after arginine); subsequently, the normalized second phase, peak after GLP-1, and peak after arginine are each expressed relative to the first phase to account for overall differences in β -cell function (e.g., between impaired glucose-tolerant and normal glucose-tolerant subjects). We are left with percentages where, for example, a 140% second phase and 60% arginine peak indicate a high second phase and a low arginine phase relative to the individual’s first phase. Thus, the spatial position in the

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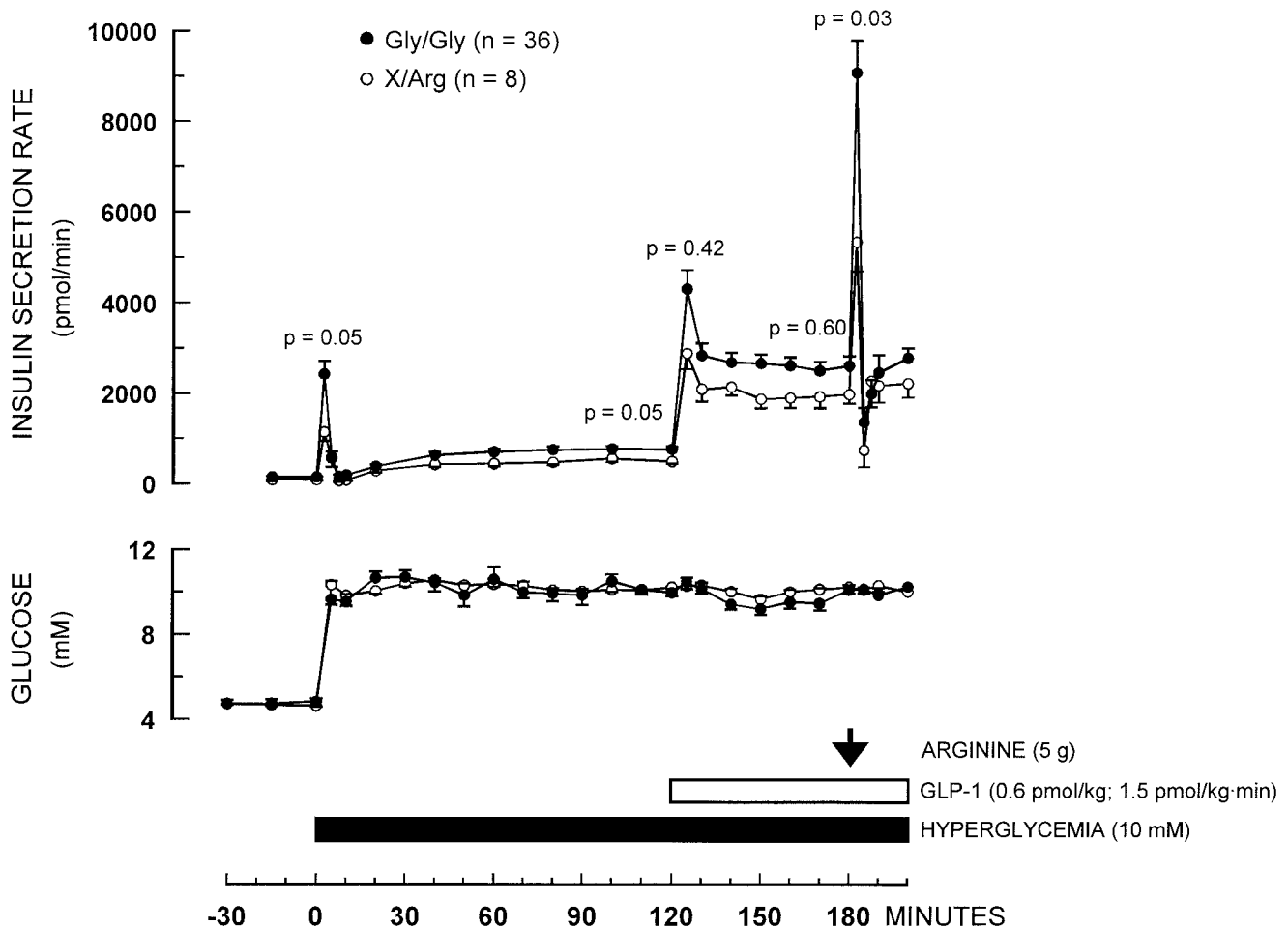


FIG. 5. Blood glucose and insulin secretion rates during a modified hyperglycemic clamp in subjects without (Gly/Gly) and with (X/Arg) the Gly972Arg polymorphism in IRS-1. The indicated *P* values were calculated at 2.5, 80–120, 125, 160–180, and 182.5 min.

three-dimensional construct (the dimensions are second phase, peak after GLP-1, and peak after arginine, all relative to first phase) is the measure for the heterogeneity of β -cell function.

In a second step, we subjected these spatial points from 80 subjects to a standard cluster analysis arbitrarily set for four clusters. This procedure clusters the 80 points (80 individuals) around four centers of gravity (Fig. 7). The individuals in one cluster have the relative height of the three secretion phases in common. We then assessed the distribution of genotypes within the four clusters. We specifically examined the frequency of polymorphisms reported to be associated with β -cell dysfunction or an increased risk for type 2 diabetes by χ^2 testing. Because the groups were small, truly significant differences could not be seen upon multiple comparison. Nevertheless, the frequency of carriers of the IRS-1 972 polymorphism was different in cluster 1 versus cluster 2 and 4 or of the CAPN10 UCSNP-43 polymorphism in cluster 1 versus cluster 4 (Table 1). A relatively low maximal secretion (arginine) appears to be associated with the IRS-1 972 polymorphism. Carriers of the G allele in CAPN10 UCSNP-43 were absent in the cluster with the relative increase in all phases relative to the first phase. The enrichment of a specific genotype may help to identify the genetic contribution of subtypes of β -cell dysfunction. We are well aware that this kind of analysis is preliminary at

best and is only intended as a potential perspective of approaching clinical characterization of β -cell dysfunction in the future. With increasing number, the statistical power

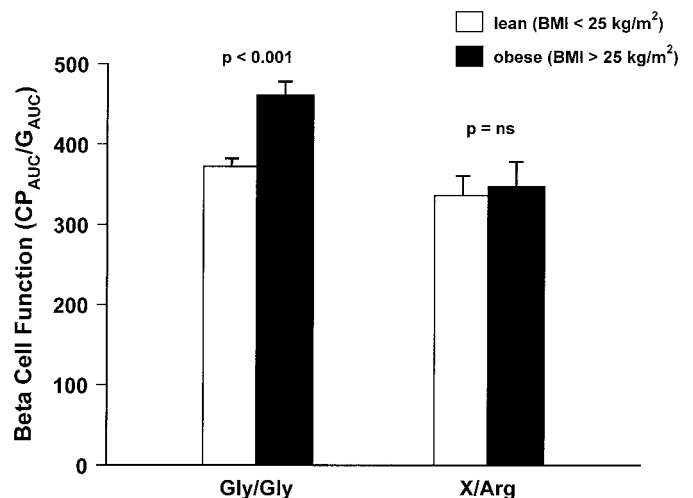


FIG. 6. Effect of obesity on β -cell function (expressed as CP_{AUC}/G_{AUC} during an OGTT) in subjects without (Gly/Gly) and with (X/Arg) the Gly972Arg polymorphism in IRS-1. Subjects within one genotype were divided into an obese and a lean group according to BMI (cutoff 25 kg/m^2). *P* = 0.078 for the interaction effect between the IRS 972 variant and obesity (BMI \geq 25 kg/m^2), ANOVA.

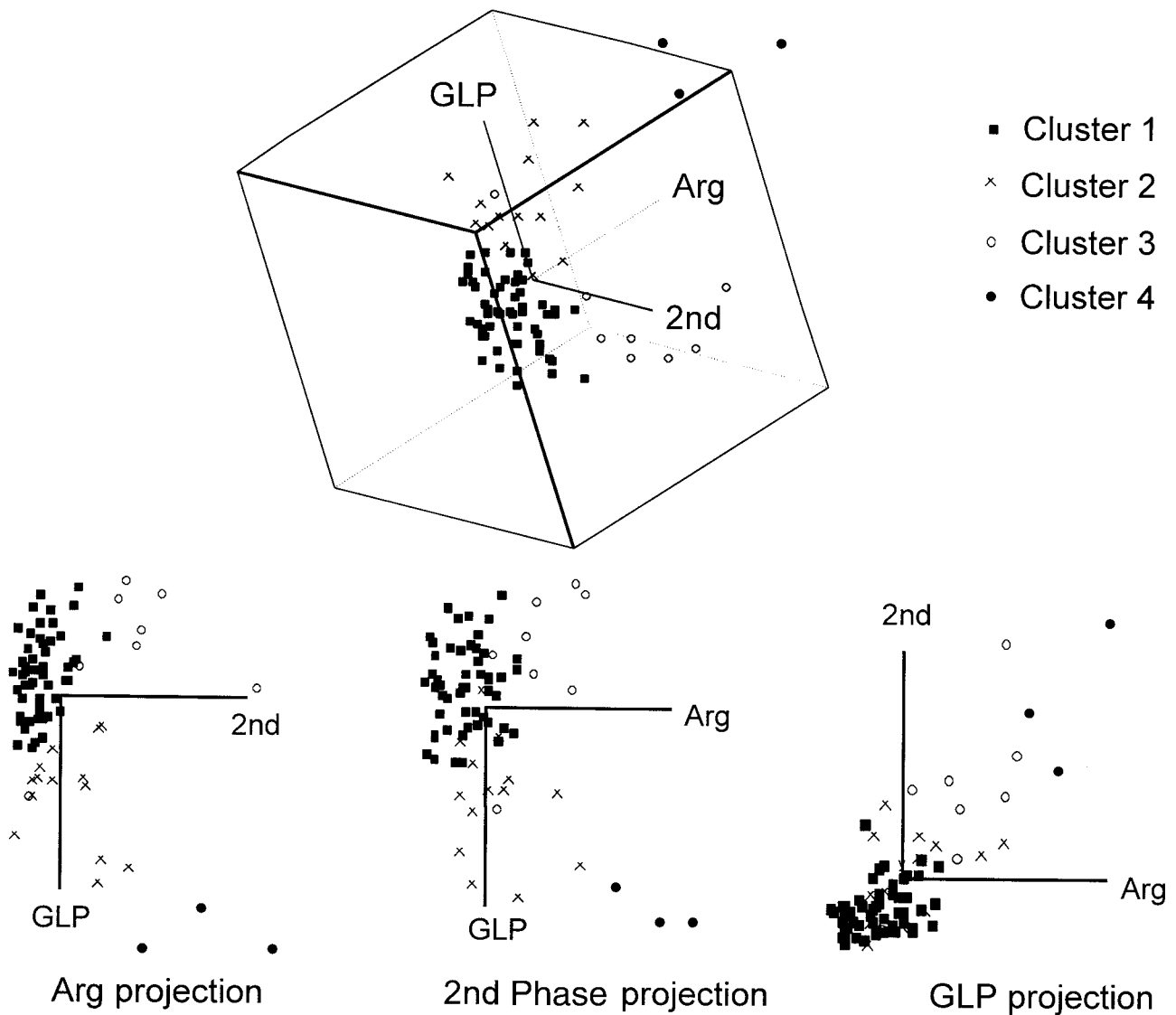


FIG. 7. Heterogeneity of insulin secretion. Each spatial point represents one individual. The *x*, *y*, and *z* coordinates indicate the insulin secretory response during the second phase, GLP-1, and arginine relative to the first phase of glucose-stimulated insulin secretion. Thus, the spatial position in the three-dimensional construct (the dimensions are second phase, peak after GLP-1, and peak after arginine relative to first phase) is the measure for the heterogeneity of β -cell function. The plot no longer contains information regarding high versus low insulin secretion. The symbol coding denotes the result of a cluster analysis performed on 80 nondiabetic subjects. The individuals in one cluster have the relative height of the three secretion phases in common (see text for more details).

will increase and more clusters and additional dimensions, for example, the proinsulin-to-insulin ratio in response to an acute stimulus as an index for proinsulin processing (34), can be introduced.

CONCLUSIONS

We presented evidence that careful clinical characterization of β -cell function will lead to the identification of

secretion defects in selected subgroups prone to develop type 2 diabetes, such as IGT, GDM, and relatives of affected patients. Moreover, as exemplified by the IRS-1 972 polymorphism, quantification of β -cell function can help to identify the contribution of a genetic variant to the biological variation of insulin secretion. In addition to categorizing insulin secretion in response to glucose, however, determining the heterogeneity of the secretory

TABLE 1
Genotype distribution among secretion clusters

| Allele | Cluster 1 (%) | Cluster 2 (%) | Cluster 3 (%) | Cluster 4 (%) | <i>P</i> * (four groups) | <i>P</i> * (selected comparison) |
|---------------------|---------------|---------------|---------------|---------------|--------------------------|----------------------------------|
| PPAR γ Prol2 | 67 | 57 | 78 | 67 | 0.52 | — |
| IRS-1 Arg972 | 25 | 11 | 18 | 0 | 0.39 | 0.058 (1 vs. 2 + 4) |
| CAPN10 UCSNP-43/G | 62 | 33 | 64 | 0 | 0.11 | 0.02 (1 vs. 4) |

PPAR γ , peroxisome proliferator-activated receptor γ . * χ^2 test.

response to different secretagogues may be a novel way to understand in vivo function and dysfunction of genetic variants allegedly contributing to β -cell dysfunction and type 2 diabetes. However, only longitudinal data will provide the final proof of our concept.

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