Insulin and Amylin Release Are Both Diminished in First-Degree Relatives of Subjects With Type 2 Diabetes

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OBJECTIVE — To determine whether first-degree relatives of individuals with type 2 diabetes, who are at high risk of subsequently developing hyperglycemia, manifest alterations in β-cell function including an alteration in the co-release of insulin and amylin.

RESEARCH DESIGN AND METHODS — In 30 first-degree relatives and 24 matched subjects with no family history of diabetes, β-cell function was measured as the intravenous glucose–induced acute insulin response (AIRg) and acute amylin response (AARg). The insulin sensitivity index (S) was quantified and used to account for the role of insulin sensitivity to modulate β-cell function (S × β-cell function).

RESULTS — Fasting plasma glucose (5.3 ± 0.1 vs. 5.1 ± 0.1 mmol/l; means ± SEM), immunoreactive insulin (IRI) (68 ± 7 vs. 57 ± 6 pmol/l) and amylin-like immunoreactivity (ALI) (5.5 ± 0.6 vs. 4.7 ± 0.7 pmol/l) were similar in relatives and control subjects, respectively.

Relative were insulin resistant compared with control subjects (S: 4.86 ± 0.63 vs. 7.20 ± 0.78 × 10^{-3} \text{ min}^{-1} \cdot \text{pmol}^{-1} \cdot 1^{-1}, P = 0.01), but their AIRg (392 ± 59 vs. 386 ± 50 pmol/l) and AARg (5.9 ± 0.9 vs. 6.1 ± 0.8 pmol/l) did not differ. When β-cell function was determined relative to insulin sensitivity, in the first-degree relatives, both AIRg (S × AIRg: 1.60 ± 0.23 vs. 2.44 ± 0.31 \times 10^{-2} \text{ min}^{-1}, P < 0.05) and AARg (S × AARg: 2.39 ± 0.35 vs. 4.06 ± 0.56 \times 10^{-4} \text{ min}^{-1}, P < 0.05) were reduced. The molar proportion of ALI to IRI was not altered in high-risk subjects (1.75 ± 0.16 vs. 1.71 ± 0.15%).

CONCLUSIONS — First-degree relatives of subjects with type 2 diabetes have diminished β-cell function at a time when they are not hyperglycemic, and this reduction affects insulin and amylin responses proportionally. Thus, an altered amylin-to-insulin ratio is not likely to identify individuals at high risk of developing type 2 diabetes.


The pathogenesis of type 2 diabetes includes reductions in insulin sensitivity and β-cell function (1). The change in β-cell function has been demonstrated to include a reduction in insulin secretion in response to glucose (2). In addition to this reduction in insulin release, it is apparent that a decrease in the release of the more recently described β-cell peptide known as amylin or islet amyloid poly peptide can also be demonstrated in subjects with type 2 diabetes (3–5).

Although the changes in insulin sensitivity and β-cell function have been demonstrated to exist once hyperglycemia is present, it has been debated as to when during the development of the disease these changes occur. A number of studies have been largely interpreted to suggest that whereas insulin resistance exists in individuals who are at high risk of developing diabetes and have normal glucose tolerance, β-cell function is not diminished (6–9), or if β-cell dysfunction is present, this dysfunction is only mild and is present only when impaired glucose tolerance exists (10,11). However, these assessments have not accounted for the fact that insulin sensitivity is an important modulator of the β-cell response to secretagogues, and therefore reduced β-cell function may easily be overlooked (12,13). Thus, when the effect of insulin sensitivity on β-cell function is accounted for, a comparable insulin response could in fact be considered inappropriately low in the face of insulin resistance. Using this approach, it is now being recognized that β-cell function is relatively decreased in some groups at high risk of developing hyperglycemia (14–18).

Amylin is a 37-amino acid peptide that is produced by the β-cell and is co-secreted with insulin in response to glucose and nonglucose secretagogues administered orally or intravenously (4,5,19,20). It is the unique constituent of the islet amyloid deposits found in the vast majority of subjects with type 2 diabetes (21–23). We and others have hypothesized that alterations in the handling of this peptide by the β-cell may underlie the propensity of diabetic individuals to deposit amyloid, resulting in a reduction in β-cell mass and the loss of β-cell secretory capacity (24). In keeping with a reduction in β-cell secretory capacity, we have observed reductions in insulin and amylin responses in individuals with impaired glucose tolerance (5). Others have found that the amylin response is increased under conditions associated with insulin resistance, such as obesity (4,25) and pregnancy (26), including in women with gestational diabetes who are at high risk for subsequently developing type 2 diabetes. Thus, changes in amylin have been observed under scenarios that are associ-
ated with an increased risk of developing type 2 diabetes.

First-degree relatives of subjects with type 2 diabetes are at increased risk of developing hyperglycemia. They therefore provide an ideal group to study to determine the magnitude of changes in insulin sensitivity and β-cell function that may be present in such high-risk individuals. Furthermore, as they are at increased risk of developing hyperglycemia, they also represent an appropriate cohort for examining whether amylin responses are altered in high-risk subjects. Therefore, we have examined a group of first-degree relatives of subjects with type 2 diabetes and a control group and quantified insulin sensitivity and both insulin and amylin responses as measures of β-cell function in these individuals to determine whether these variables are altered in these high-risk subjects. In the process, we also examined whether differential alterations in insulin and amylin responses may provide an additional useful marker for subjects at high risk of developing type 2 diabetes.

## RESEARCH DESIGN AND METHODS

### Subjects

The groups consisted of 30 (9 males/21 females) individuals with at least one first-degree relative with a known diagnosis of type 2 diabetes and 24 (14 males/10 females) subjects who had no known family history of type 2 diabetes. No subject was taking medications that are known to affect glucose metabolism. All subjects gave written informed consent before participating in the study, which had been reviewed and approved by the Human Subjects Review Committee at the University of Washington.

### Study methods

Weight and height were measured and used to calculate BMI as weight (kg)/height² (m²).

After a 10-h overnight fast, a tolbutamide-modified frequently sampled intravenous glucose tolerance test was performed to quantify insulin sensitivity and the first-phase insulin and amylin responses. After basal sampling, glucose (11.4 g/m²) was administered intravenously over 60 s, and 20 min later, tolbutamide (125 mg/m²) was injected intravenously over 30 s. Blood samples were obtained 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, and 240 min after glucose administration. The administration of tolbutamide and the prolonged sampling schedule served to improve the ability to identify the parameters (27) when the glucose and insulin data were analyzed using the minimal model of glucose kinetics developed by Bergman et al. (28). All samples were assayed for glucose and insulin, whereas only the basal samples and those drawn up to 10 min after glucose injection were assayed for amylin.

### Assays

All blood samples were drawn into tubes containing EDTA, kept on ice before separation, and subsequently stored at −70°C before being assayed. Plasma glucose was measured by an automated glucose oxidase method. Plasma immunoreactive insulin (IRI) was measured by a radioimmunoassay that has inter- and intra-assay coefficients of variation of 12 and 8%, respectively. The antibody used in this assay cross-reacts fully with intact proinsulin and its conversion intermediates. Plasma amylin-like immunoreactivity (ALI) was quantified using a two-site enzyme-linked immunoassay system developed by Amylin Pharmaceuticals (San Diego, CA) using antibodies F002 and F025 (29).

This assay measures both glycosylated and nonglycosylated forms of the peptide (29,30). It has inter- and intra-assay coefficients of variation of <15 and <10%, respectively, with a minimum detectable concentration of 1.6 pmol/l. IRI was measured in duplicate, whereas glucose and ALI were measured in triplicate.

### Calculations and statistical analysis

Using the minimal model of glucose kinetics, insulin sensitivity was quantified as the insulin sensitivity index (S_I) and glucose effectiveness at basal insulin (S_{G0}) (28). Glucose effectiveness at zero insulin [GEZI = S_I - (S_I × fasting IRI)] was calculated as a measure of insulin-independent glucose disposal (31). The intravenous glucose-induced acute insulin (AIRg) and acute amylin (AARg) responses were calculated as the mean incremental response above basal from the samples drawn during the first 10 min after intravenous glucose administration. The percentile ranking for each individual’s product of S_I and AIRg (disposition index) was determined using the formula

$$Z_a = \left[ \ln(S_I \times AIR_g) + 3.802 \right] / 0.5613$$

which was originally determined by our group in a cohort of 93 apparently healthy subjects (13).

The glucose disappearance constant was calculated as the slope of the regression line relating the natural log of the glucose concentration versus time for samples drawn between 10 and 19 min after glucose administration.

Statistical analysis was performed using Statview SE + Graphics (Abacus Concepts, Berkeley, CA). Data are presented as means ± SEM. Comparisons between groups were done using an unpaired Student’s t test, except when variables were non-normally distributed, in which case the Mann-Whitney U test was performed. Correlations were performed by linear regression. A P value of <0.05 was considered significant.

## RESULTS

### Subject characteristics and fasting measures

Age, BMI, fasting plasma glucose, IRI, and ALI did not differ significantly between the 30 first-degree relatives and 24 control subjects (Table 1).

| Table 1—Demographic characteristics and fasting plasma glucose, IRI, and ALI concentrations in first-degree relatives of type 2 diabetic subjects and in control subjects |
|----------------|----------------|
|                | First-degree relatives | Control subjects |
| n              | 30                        | 24                      |
| Age (years)    | 39.8 ± 1.7                | 42.0 ± 3.1              |
| BMI (kg/m²)    | 29.2 ± 1.2                | 26.7 ± 0.8              |
| Fasting glucose (mmol/l) | 5.3 ± 0.1               | 5.1 ± 0.1               |
| Fasting IRI (pmol/l) | 68 ± 7                  | 57 ± 6                  |
| Fasting ALI (pmol/l) | 5.5 ± 0.6               | 4.7 ± 0.7               |

Data are means ± SEM.
Insulin and amylin release in first-degree relatives

Insulin sensitivity, β-cell function, and intravenous glucose tolerance

S₁, which was quantified using the frequently sampled intravenous glucose tolerance test and minimal model of glucose kinetics, indicated that the first-degree relatives were insulin resistant. In the relatives, S₁ was 4.86 ± 0.63 × 10⁻³ min⁻¹ · pmol⁻¹·l⁻¹·min⁻¹, whereas in the control subjects, it was 7.20 ± 0.78 × 10⁻³ min⁻¹ · pmol⁻¹·l⁻¹·min⁻¹ (P < 0.05). The first-phase IRI (AIRᵣ) and ALI (AARᵣ) secretory responses, determined over the first 10 min after intravenous glucose administration, did not differ in absolute terms between the two groups. In the first-degree relatives, AIRᵣ was 392 ± 59 pmol/l and AARᵣ was 5.9 ± 0.9 pmol/l, whereas in the control subjects, these measures were 386 ± 50 and 6.1 ± 0.8 pmol/l, respectively.

We determined whether the β-cell responses were appropriate by calculating the product of S₁ and the acute insulin and amylin responses (S₁ × AIRᵣ and S₁ × AARᵣ). When the data were examined in this way, it was clear that the β-cell responses in the first-degree relatives were not normal but were proportionally reduced. In these high-risk subjects, S₁ × AIRᵣ was 1.60 ± 0.23 × 10⁻² min⁻¹, compared with 2.44 ± 0.31 × 10⁻² min⁻¹ in the control subjects (P < 0.05). Similarly, when β-cell function was quantified as S₁ × AARᵣ, this measure was also significantly lower in the first-degree relatives, being 2.39 ± 0.35 × 10⁻³ min⁻¹ versus 4.06 ± 0.56 × 10⁻³ min⁻¹ in the control subjects (P < 0.05).

To determine the distribution of the disposition index (S₁ × AIRᵣ) in the two groups of subjects, we determined the percentile score for each subject based on the Z₀ derived from S₁ and AIRᵣ values in a healthy population (13). As illustrated in Fig. 1, although there was considerable overlap in the percentile scores, the distribution of percentile scores differed between the two groups of subjects, with 21 of the 30 relatives falling below the 50th percentile compared with 12 of the 24 control subjects. Thus, the mean percentile score for the first-degree relatives was 29.6 ± 6.0% compared with 47.7 ± 6.7% in the control subjects (P < 0.05).

As would be expected in a group of individuals in whom both insulin sensitivity and insulin secretion are diminished, intravenous glucose tolerance, determined as the glucose disappearance constant, tended to be reduced in the first-degree relatives compared with the control subjects (1.64 ± 0.12 vs. 1.84 ± 0.12%/min; P = 0.08). GEZI, a measure of insulin-independent glucose disposal, did not differ significantly between the two groups, being 0.016 ± 0.001 min⁻¹ in the first-degree relatives and 0.020 ± 0.002 × 10⁻² min⁻¹ in the control subjects (P > 0.1).

Relationship between amylin and insulin responses

Using linear regression, we assessed whether the relationship between fasting ALI and IRI levels was different between first-degree relatives and control subjects. As illustrated in Fig. 2A, these fasting values were linearly correlated in the whole population (r = 0.42; P < 0.005). When the two groups were examined independently, the relationship was not different between first-degree relatives (r = 0.38; P < 0.05) and control subjects (r = 0.49; P < 0.05). The ratio of fasting ALI to fasting IRI did not differ between the two groups, being 9.5 ± 1.4% in the first-degree relatives versus 8.7 ± 1.1% in the control subjects.

We also determined whether the stimulated amylin (AARᵣ) and insulin (AIRᵣ) responses were related in the whole cohort and when they were subdivided into the two groups. As illustrated in Fig. 2B, there was a great degree of overlap in absolute AARᵣ and AIRᵣ between individuals in both groups. For the whole study group, these two responses were strongly linearly related (r = 0.89; P < 0.0001), and the results were not different for the first-degree relatives (r = 0.91; P < 0.0001) and control subjects (r = 0.86; P < 0.0001). Again, the relationship between these two parameters did not differ between the relatives and control subjects. Each individual’s AIRᵣ and AARᵣ were used to calculate the molar proportions of these responses for the two groups. The AIRᵣ/AARᵣ molar ratio did not differ between the first-degree relatives in whom it was 1.75 ± 0.16% compared with 1.71 ± 0.15% in the control subjects.

CONCLUSIONS — Many studies examining the pathogenesis of type 2 diabetes used absolute measures of insulin sensitivity and insulin responses and concluded that insulin resistance is the primary defect, especially when glucose tolerance is still normal (6–11). However, based on findings in healthy control subjects, the concept of a feedback loop between the insulin-sensitive tissues and the
islet has emerged (12,13,17,32). Thus, the relationship between insulin sensitivity and H9252-cell function is hyperbolic, and the product of insulin sensitivity and H9252-cell function, known as the disposition index, is a constant.

Using the disposition index as a measure of H9252-cell function, first-degree relatives had responses that ranged between the 1st and 88th percentiles, with 70% of the first-degree relatives falling below the mean (50th percentile), whereas 12 control subjects were above and 12 were below the 50th percentile. Because calculation of the percentile score with this formula is independent of differences in insulin assays, provided both high and low insulin values are reliably measured, it is applicable to populations studied with different insulin assays. The utility of this formula has also been demonstrated by Elbein et al. (33), who demonstrated the heritability of the disposition index to be 70% in subjects with normal glucose tolerance who have a sibling with type 2 diabetes. These data raise the interesting possibility as to whether assessment of the percentile score for individuals at high risk of developing type 2 diabetes could possibly be used as a tool for predicting subjects who are at risk. Such a possibility will require longitudinal studies of such high-risk individuals.

It is of interest that in the first-degree relatives, impairments of both insulin sensitivity and H9252-cell function existed, yet these high-risk subjects did not have diabetes. The concept that these parameters may be reduced without a marked deterioration in glucose tolerance is apparent from studies of other groups at high risk for diabetes who have abnormalities in both insulin secretion and insulin sensitivity at a time when they have relatively normal glucose tolerance (14,17,18). These findings highlight the fact that more marked alterations in both insulin sensitivity and β-cell function must exist for impaired glucose tolerance or diabetes to exist, and emphasize the importance of insulin-independent glucose disposal to glucose tolerance (34,35).

Amylin is a secretory product of the β-cell that is typically co-released with insulin (36). As is the case with insulin, in healthy subjects, the relationship between insulin sensitivity and amylin levels is hyperbolic (37). Thus, by taking into account differences in insulin sensitivity, we were also able to demonstrate a reduction in the amylin response in the first-degree relatives. It could be argued that it is not optimal to assess the amylin response in relationship to insulin sensitivity because insulin sensitivity may not be a modulator of amylin release by the β-cell. However, because insulin and amylin are packaged together in β-cell secretory granules (38) and are typically co-released from the regulated secretory pathway in response to classic β-cell secretagogues (36), because amylin levels have been shown to be increased in insulin-resistant states such as obesity (4,5,25), and because the relationship between amylin and insulin sensitivity is hyperbolic (37), it seems reasonable to assume that the effect of insulin sensitivity to modulate the release of insulin-containing granules would apply to amylin as well.

In addition, the molar proportion of amylin to insulin, both fasting and stimulated, was not different between high-risk and control subjects. The higher ratio during fasting is because amylin clearance is slower than that of insulin (25). Our findings differ from that of Kautzky-Willer et al. (26), who reported that pregnancy was associated with an increase in this ratio. It must be noted that our approach does not allow us to discern small differences that may be identifiable if we were sampling directly at the level of the pancreas. That such differences might occur is supported not only by the study of

Figure 2—Relationship between (A) the fasting values for IRI and ALI and (B) AIRg and AARg in first-degree relatives of subjects with type 2 diabetes (○) and apparently healthy control subjects (□). The linear correlation for the whole cohort for both sets of variables is illustrated (fasting: r = 0.42; P < 0.005; stimulated: r = 0.89; P < 0.0001). It is apparent that this relationship does not differ between groups.
pregnant women (26) but also from models in which more proximal sampling has been performed in vitro and in situ (39–41). However, based on our data, the circulating proportion of amylin and insulin probably will not be useful as a clinical marker of β-cell dysfunction.

The present finding of reductions in both insulin and amylin responses in high-risk subjects suggests that the early changes in β-cell function are fairly generalized, affecting the release of both these peptides. Because amylin is the unique peptide constituent of the islet amyloid deposits that are typically observed in individuals with type 2 diabetes (21–23), it has been suggested that increased amylin release may play a role in the development of these deposits. The present findings suggest the possibility that in high-risk individuals, the absolute amylin responses and the proportion relative to insulin may not differ from those in low-risk subjects. However, it is also clear from this study that, at the time we studied these subjects, the relative responses were reduced. Thus, the question is still open as to whether before the stage at which we studied these subjects the absolute output of amylin was increased and contributed to the initiation of amyloid fibril formation.

The present study was performed in groups that were matched for BMI and age, two parameters recognized to affect insulin sensitivity (42,43). However, other factors such as body fat distribution that may affect insulin sensitivity were not assessed. Although such parameters may have differed between the two groups, we do not believe these parameters would have affected the outcome of these studies because they affect insulin sensitivity, which we directly quantified, and related to parameters of β-cell function. In our assessments, we used an insulin assay that also recognizes proinsulin. Proinsulin levels were shown to be disproportionately increased in subjects with type 2 diabetes (44–47), and a small increase was also demonstrated in individuals at high risk of developing the disease (48,49). Whereas we have quantified the insulin response shortly after granule exocytosis, any effect of the slower rate of clearance of proinsulin would have made it more difficult to demonstrate a difference between first-degree relatives and control subjects. Thus, we believe that the findings of the present study highlight that first-degree relatives of subjects with a history of type 2 diabetes are both insulin resistant and have impaired insulin and amylin responses to this insulin resistance.

In summary, we found that apparently healthy first-degree relatives of subjects with type 2 diabetes clearly have defects in insulin sensitivity and β-cell function. The latter abnormality involves reductions in first-phase insulin and amylin release in response to glucose stimulation. However, these reductions are not evident from the absolute responses and require a simultaneous assessment of insulin sensitivity. When the modulating effect of insulin sensitivity on β-cell function is taken into account, the defect is obvious. Finally, we have found that despite the differences in insulin sensitivity and the reduction in β-cell function, as determined by the insulin and amylin responses, the release of these two peptides is proportionate. Therefore, determination of the relative proportions of amylin and insulin is unlikely to be an additional useful marker of β-cell function in humans.

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References
15. Larsson H, Ahren B: Failure to adequately adapt reduced insulin sensitivity with increased insulin secretion in women with impaired glucose tolerance. Diabetologia 39:1099–1107, 1996

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