Hyperproinsulinemia and Proinsulin-to-Insulin Ratios in Swedish Middle-aged Men: Association with Glycemia and Insulin Resistance but Not with Family History of Diabetes

V. Grill,1,2 B. Dinesen,3 S. Carlsson,4 S. Efendic,1 O. Pedersen,3 and C.-G. Östenson1

Elevated proinsulin and proinsulin/insulin ratios are features of abnormal β-cell function in type 2 diabetes. The participation of genetic factors is disputed. The authors wished to investigate relations between family history of diabetes on one hand and proinsulin as well as proinsulin/immunoreactive insulin ratios on the other. A large, population-based sample of Swedish men aged 35–54 years in 1992 was studied. Subjects without known diabetes were selected either to have a strong family history of diabetes (n = 1,619) or no history of the disease (n = 1,495). An oral glucose tolerance test detected 172 subjects with impaired glucose tolerance and 55 subjects with previously unknown diabetes according to World Health Organization 1985 criteria. In multiple regression analysis, fasting levels of proinsulin and proinsulin/insulin ratios were positively associated both with the 2-hour glucose level (as a continuous variable) and with obesity, whereas a negative association was found with birth weight. No association was found with family history of diabetes or with chronologic age. These findings indicate that elevated proinsulin and proinsulin/insulin ratios are secondary to increased demands on β-cell secretion induced by hyperglycemia and insulin resistance with no discernible influence of family history of diabetes. Am J Epidemiol 2002;155:834–41.

Received for publication November 14, 2000, and accepted for publication January 11, 2002.

Abbreviations: BMI, body mass index; IGT, impaired glucose tolerance; IRI, immunoreactive insulin; OGTT, oral glucose tolerance test; P/IRI, proinsulin/immunoreactive insulin; WHO, World Health Organization.

1 Department of Molecular Medicine, Karolinska Institutet, Stockholm, Sweden.
2 Department of Medicine, Endocrinology Section, University Hospital of Trondheim, Trondheim, Norway.
3 Steno Diabetes Center, Gentofte, Denmark.
4 Division of Epidemiology, Karolinska University Hospital, Stockholm, Sweden.

Correspondence to Prof. Valdemar Grill, Department of Medicine, Endocrinology Section, University Hospital of Trondheim, N-7006 Trondheim, Norway (e-mail: valdemar.grill@medisin.ntnu.no).
Proinsulin and Family History of Diabetes

population with subjects who had a strong family history of diabetes to provide one half of the study population. The results obtained from these subjects were then to be contrasted with the other, age-matched half of the study population consisting of subjects with no known relatives with diabetes. Results have been reported on the diabetogenic influence of family history of diabetes (23), obesity (24), and birth weight (25) in this population.

The aim of this study was to analyze the influence of family history of diabetes on fasting levels of proinsulins as well as on P/IRI ratios, taking into account the possibly confounding influence exerted by age, level of glycemia, obesity, and birth weight.

MATERIALS AND METHODS

Subjects

This investigation was designed as a population-based cross-sectional study. It was approved by the Ethics Committee of the Karolinska Hospital. The study population consisted of a sample of men who were aged 35–54 years in 1992. (Since data were assembled between 1992 and 1994, the actual age span of subjects at the time point of data collection was 35–56 years.) All subjects of the appropriate age who lived in the four municipalities (Sigtuna, Tyresö, Värmdö, and Upplands Väsby) were included in the initial investigations. All four municipalities belong to the outer Stockholm area and are located between 20 and 40 km from the city center. The subjects to be studied were identified through the continuously updated population registry maintained by the Stockholm County Council, which includes all inhabitants of the county.

An outline of the selection procedure is given in figure 1. Sampling of subjects was performed by two sequential procedures. In the first procedure, a short questionnaire was sent to all men in the appropriate age group who lived in the four municipalities. The questionnaire asked about country of birth and presence of diabetes in the subject and among

FIGURE 1. Study design of a large, population-based study of Swedish men aged 35–54 years in 1992. WHR, waist-hip ratio.

Am J Epidemiol Vol. 155, No. 9, 2002
his relatives. Answers were obtained from 10,236 (79 percent) of the 12,952 men who received the questionnaire. The answers revealed that 258 men (2.5 percent) knew that they had diabetes and 212 (2.1 percent) were born outside Sweden. These subjects were excluded from the remainder of the study.

From the remaining questionnaires, we identified 2,106 men with a family history of type 2 diabetes specified as follows: known diabetes in at least two second-degree relatives (grandparent, uncle, or aunt) or in at least one first-degree relative in the generation of the proband (sister or brother) or the preceding generation (father, mother). Subjects who reported having children with diabetes were not included in the family history group because of the likelihood that their family history indicated links to type 1 rather than to type 2 diabetes. We furthermore identified 3,329 subjects who did not have first- or second-degree relatives (including children) or cousins with known diabetes. These subjects constituted the group without family history. We excluded from further investigations 1,531 (15.0 percent) men who did not fit either the family history or the nonfamily history category and 2,800 men (27.4 percent) who did not give complete answers on family history.

In the second procedure, all 2,106 men who had given complete and positive information on family history were contacted by telephone and asked to participate in further investigations. Along with the subjects with family history, we contacted in the same manner 2,424 of those subjects without family history. The latter subjects were randomly selected within the 5-year age group of each participating subject with a family history. In total, 3,162 (69.8 percent) of those contacted agreed to participate. The participation rate for subjects with a family history of diabetes was higher (76.9 percent) than in those without a family history of diabetes (62.2 percent).

The investigations took place at four local health care centers, one for each of the participating municipalities. The subjects to be studied were reported to the local health care center of their community in the morning (between 7:00 and 8:30 a.m.) after an overnight fast (starting at 10 p.m.). Subjects were instructed to abstain from vigorous exercise the evening before and on the morning of the investigations. Smokers were encouraged to abstain from smoking on the morning of the investigations. When subjects arrived at the health care center, they were asked to verify the information given by the subjects in the questionnaire on family history. As a result of this verification, 33 subjects were found not to fit the family history or nonfamily history category and were therefore excluded from further studies. In all others, weight, height, and waist-hip ratios were measured with the subjects wearing light indoor clothing and no shoes. A nurse measured blood pressure with the subjects in the supine position.

Subsequent to these measures, subjects underwent a 75-g OGTT according to World Health Organization (WHO) criteria (26). Venous blood samples were secured before and 120 minutes after the ingestion of glucose. Plasma samples were obtained after centrifugation and stored at −20°C until assays of glucose, immunoreactive insulin (IRI), and proinsulins. During the OGTT, the subjects were comfortably seated in calm surroundings. A detailed questionnaire, which included eating habits, physical activity, and social conditions, was filled out at that time.

One person did not finish the OGTT. The final study population thus contained 3,128 subjects. Of these, 2,237 (71.5 percent) were able to report on birth weight. (Birth weight had already been asked for in the invitation letter preceding the investigations, thereby giving the subjects time for recollection and/or consultation with mothers.)

Classifications

Impaired glucose tolerance (IGT) and diabetes were defined according to WHO criteria of 1985 (26). Thus, according to the results of the OGTT, diabetes was defined as a 2-hour plasma glucose level above 11.0 mmol/liter and IGT as a level between 7.8 and 11.0 mmol/liter. Further, a diagnosis of diabetes was also made if fasting plasma glucose exceeded 7.8 mmol/liter. The presence of overweight was arbitrarily defined as a body mass index (BMI) of 28 kg/m² or more.

Assays

Levels of plasma glucose were assayed in duplicate by using a glucose oxidase method and a Yellow Spring Glucose Analyzer (Yellow Springs, Inc., Yellow Springs, Ohio).

IRI was assayed by radioimmunoassay, using our own antibodies, human insulin as a standard, and charcoal addition to separate antibody-bound and free IRI (27). Proinsulins cross-react in this assay by about 80 percent.

Proinsulin was determined by enzyme-linked immunosorbent assay with a broad specificity comprising, in addition to intact proinsulin, the four proinsulin conversion intermediates, reacting with 65–99 percent efficiency relative to intact proinsulin. The limit of detection was 0.25 pmol/liter, and the total interassay coefficient of variation was 7.6–5.3 percent at 1.1–8 pmol/liter (28). The assay was performed on samples stored for 3 years at −20°C. These samples had previously been thawed once for the assay of IRI. For ascertainment of whether thawing affected measurements of proinsulins, the results of the assay were compared in 20 samples that had previously been thawed and 20 samples that had not been thawed, with all samples being obtained from the same subjects at the same occasion. To obtain large interindividual variation in levels of proinsulins, we chose samples from subjects who displayed different degrees of glucose tolerance. The mean level of proinsulins in previously thawed samples was 10.17 pmol/liter, and the range was 2.6–30 pmol. Corresponding values for unthawed samples were a mean of 9.97 and a range of 2.5–29 pmol/liter. The mean difference, 0.20 pmol/liter between thawed and unthawed samples, was not significant.

For participating subjects, we obtained complete information on levels of proinsulins for 3,113 (99.6 percent) of the subjects.
Expression of results

Results are expressed as mean and standard deviation or standard error. Significance testing was performed by the Student t test. A probability of less than 0.05 was considered significant. Linear regression analysis was performed in which the two dependent variables were the log-transformed proinsulins and the proinsulins to IRI ratio. Independent variables were family history of diabetes, age, body mass index, birth weight, 2-hour plasma glucose levels, and 0- and 2-hour serum insulin levels. The independent variables were inserted in univariate and multivariate analyses. The analyses were performed using the SAS statistical package (SAS/STAT Software, version 6.12, SAS Institute, Inc., Cary, North Carolina).

RESULTS

Glucose tolerance of the study population

Using the WHO 1985 criteria (28), we identified 55 subjects with diabetes and 172 subjects with IGT. Proinsulins could be measured in all subjects, except one with diabetes. Thus, the total number of subjects with diabetes in this study was 54.

Influence of family history of diabetes on BMI, blood pressure, fasting glucose, and IRI levels

Family history of diabetes was associated with higher BMI in subjects with normal glucose tolerance but had no significant influence in subjects with IGT or diabetes (table 1). Likewise, family history was associated with higher fasting glucose as well as fasting insulin in subjects with normal glucose tolerance but exerted no significant influence in subjects with IGT or diabetes. In subjects with diabetes, diastolic blood pressure was lower in those with than in those without a family history of diabetes.

Levels of proinsulins

Fasting levels of proinsulins increased progressively with worsening of glucose tolerance. Levels of proinsulin were thus lowest in subjects with normal glucose tolerance, intermediate in subjects with IGT, and highest in subjects with diabetes (table 2). All glucose tolerance effects on proinsulins (normal glucose tolerance vs. IGT and IGT vs. diabetes) were significant at $p < 0.001$. In obese subjects (BMI $\geq 28$ kg/m$^2$), levels of proinsulins were increased at all degrees of glucose tolerance compared with subjects with lower BMI.

In contrast to the obvious effects of glucose tolerance and obesity, the presence of family history only marginally increased mean levels of proinsulins in normal glucose tolerance and IGT and not at all in diabetes (table 2).

P/IRI ratios

Similar to proinsulins, the fasting P/IRI ratios increased with worsening of glucose tolerance (table 2). The effects on P/IRI were significant for normal glucose tolerance versus IGT ($p < 0.05$ or less for all four groups), but for IGT versus diabetes the effects were significant only when groups with family history of diabetes and BMI of 28 kg/m$^2$ or more ($p < 0.004$) were compared. As for proinsulins, the P/IRI ratios increased with increasing BMI, except in overt diabetes. A family history of diabetes did not significantly influence the P/IRI ratios (table 2).

Univariate analysis: proinsulins and P/IRI ratios

In univariate analysis of all subjects, the levels of proinsulins were positively associated with family history of diabetes, BMI, chronologic age, and fasting and 2-hour plasma glucose levels and negatively associated with birth weight (table 3). The effects of family history of diabetes were, however, small (explained variance = 0.007, i.e., less than 1 percent) as was that of birth weight (explained variance = 0.007). Similarly to fasting levels of proinsulins, in univariate analysis, the P/IRI ratio was significantly associated with family history of diabetes (table 3).

Multivariate analysis: all subjects

In multivariate analysis, the effect of family history of diabetes on levels of proinsulins was still significant when age and BMI were entered into the model but was lost when either fasting or 2-hour plasma glucose was added to the model (table 4). In multivariate analysis, BMI, fasting glucose, and birth weight contributed significantly to the model, whereas family history of diabetes and chronologic age did not. The explained variance was 0.288. Similar results were obtained when the 2-hour plasma glucose levels during OGTT replaced the variable of fasting plasma glucose (results not shown).

For P/IRI ratios, the effect of family history of diabetes was still significant when age was brought into the model. Significance was lost when BMI was added ($p < 0.1$, table 4). Any tendency for an effect disappeared when fasting glucose was brought into the model (table 4). The P/IRI ratio was positively associated with BMI and fasting or 2-hour plasma glucose levels and negatively associated with birth weight ($p < 0.05$ or less for each factor). However, the explained variance in this model (0.166) was less than that for levels of proinsulins as dependent variable.

Multivariate analysis: separate for normal glucose tolerance, IGT, and diabetes

It seemed possible that differences could exist in the regulation of levels of proinsulins at different degrees of glucose tolerance. Therefore, the results were analyzed in multiple regression analysis separately for persons with normal glucose tolerance, with IGT, and with diabetes. In regression models with fasting levels of proinsulins as the dependent variable and family history of diabetes, fasting or 2-hour glucose, chronologic age, and birth weight as independent variables, the significant factors contributing to the model were (in subjects with normal glucose tolerance) BMI, fasting or 2-hour plasma
glucose levels, and birth weight. Significant factors in IGT subjects were BMI and fasting or 2-hour glucose levels but not birth weight. When fasting insulin was brought into the model, the significance of BMI was lost in IGT and diabetes but not in normal glucose tolerance. The same results were obtained when P/IRI was the dependent variable. In none of the three classes of glucose tolerance did family history of diabetes or chronologic age contribute to the regression models.

### TABLE 1. Age, body mass index, blood pressure, glucose, and immunoreactive insulin levels in relation to family history of diabetes, Sweden, 1992–1994

<table>
<thead>
<tr>
<th>Parameter and family history of diabetes</th>
<th>Normal glucose tolerance (mean (SD))</th>
<th>Impaired glucose tolerance (mean (SD))</th>
<th>Diabetes (mean (SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1,418–1,425</td>
<td>59–60</td>
<td>10</td>
</tr>
<tr>
<td>Yes</td>
<td>1,451–1,462</td>
<td>11–112</td>
<td>44</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>46.6 (4.9)</td>
<td>48.1 (4.2)</td>
<td>48.9 (4.4)</td>
</tr>
<tr>
<td>Yes</td>
<td>46.5 (4.9)</td>
<td>47.5 (4.9)</td>
<td>48.7 (4.3)</td>
</tr>
<tr>
<td>BMI† (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>26.2 (3.4)*</td>
<td>29.57 (5.3)</td>
<td>28.8 (4.8)</td>
</tr>
<tr>
<td>Yes</td>
<td>25.5 (3.2)</td>
<td>28.8 (3.8)</td>
<td>31.1 (5.3)</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>123.9 (14.4)</td>
<td>135.5 (18.2)</td>
<td>136.8 (13.9)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>124.7 (14.0)</td>
<td>134.5 (18.7)</td>
<td>133.5 (16.2)</td>
</tr>
<tr>
<td>Fasting glucose (mmol/liter)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>4.64 (0.58)</td>
<td>5.28 (0.78)</td>
<td>8.13 (4.28)</td>
</tr>
<tr>
<td>Yes</td>
<td>4.74 (0.61)*</td>
<td>5.44 (0.67)</td>
<td>7.94 (3.24)</td>
</tr>
</tbody>
</table>

### TABLE 2. Fasting levels of proinsulins and proinsulin/immunoreactive insulin ratios in relation to family history of diabetes, body mass index, and glucose tolerance, Sweden, 1992–1994

<table>
<thead>
<tr>
<th>Family history of diabetes</th>
<th>BMI (kg/m²)</th>
<th>No. of subjects</th>
<th>Mean (SD)†</th>
<th>Mean (SD)†</th>
<th>No. of subjects</th>
<th>Mean (SD)†</th>
<th>No. of subjects</th>
<th>Mean (SD)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proinsulin (mmol/liter)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>&lt;28</td>
<td>7.06 (7.17)</td>
<td>1,143</td>
<td>12.36 (9.28)*</td>
<td>26</td>
<td>26.21 (28.15)**</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>&lt;28</td>
<td>7.34 (5.37)</td>
<td>1,100</td>
<td>13.22 (8.64)*</td>
<td>51</td>
<td>22.45 (26.87)**</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>≥28</td>
<td>12.63 (12.00)***</td>
<td>278</td>
<td>19.95 (11.07)<em>.</em>**</td>
<td>34</td>
<td>50.78 (37.80)<strong>.</strong>*</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>≥28</td>
<td>13.48 (11.38)***</td>
<td>358</td>
<td>21.31 (17.42)<em>.</em>**</td>
<td>61</td>
<td>49.83 (43.12)<strong>.</strong>*</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>P/IRI†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>&lt;28</td>
<td>0.065 (0.036)</td>
<td>1,141</td>
<td>0.103 (0.076)*</td>
<td>25</td>
<td>0.148 (0.084)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>&lt;28</td>
<td>0.067 (0.035)</td>
<td>1,098</td>
<td>0.094 (0.047)*</td>
<td>51</td>
<td>0.131 (0.094)</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>≥28</td>
<td>0.085 (0.070)***</td>
<td>278</td>
<td>0.110 (0.047)<em>.</em>**</td>
<td>34</td>
<td>0.137 (0.042)</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>≥28</td>
<td>0.088 (0.051)***</td>
<td>358</td>
<td>0.108 (0.057)<em>.</em>**</td>
<td>61</td>
<td>0.207 (0.028)<strong>.</strong>*</td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05 or less for effect versus normal glucose tolerance.
** p < 0.05 or less for effect versus impaired glucose tolerance.
*** p < 0.05 or less for effect versus body mass index (BMI) < 28.
† SD, standard deviation; P/IRI, proinsulin/immunoreactive insulin.
DISCUSSION

The main aim of this large, population-based study was to test for an influence of family history of diabetes on proinsulins and P/IRI ratios. The results conclusively demonstrate that there is no such influence in this population. Other studies have reported on this issue, with conflicting results (6–10). There are, however, reasons why, in our view, our findings should be assigned special importance.

First, our study was designed to increase the sensitivity of detection of a possible effect of family history of diabetes. Sensitivity was increased by enriching for a strong history of diabetes in one half of the population and then contrasting results in this half of the population with those who did not know of any diabetes in relatives. Such an approach increases the likelihood of detecting even a minor influence of family history of diabetes. The possibility of detection is further enhanced by the demonstration of a strong diabeticogenic influence of family history of diabetes in our study population. Hence, family history of diabetes, adjusted for other risk factors, was associated with a 4.4 times increased risk of diabetes and an 1.8-fold increased risk of IGT (23).

Second, we report on a large, population-based study in which all subjects performed an OGTT. This allows for a proper analysis of glucose tolerance as a factor influencing levels of proinsulins and P/IRI ratios not only for diabetes and IGT, as previously done, but also for subjects with normal glucose tolerance. We find that 2-hour plasma glucose levels during OGTT correlate positively with levels of proinsulins and with P/IRI ratios over the entire range of recorded glucose levels. To our knowledge, such a degree of correspondence has not previously been reported. A family history of diabetes is likewise associated with higher plasma glucose levels over the entire range of 2-hour plasma glucose values (23). Correction for the glucose tolerance factor is thus necessary for proper interpretation of any effect of family history on levels of proinsulins and P/IRI ratios.

TABLE 3. Effects on proinsulin and proinsulin/immunoreactive insulin of family history of diabetes, age, body mass index, birth weight, and glucose at 0 and 2 hours, Sweden, 1992–1994

<table>
<thead>
<tr>
<th>Variables included in the model*†</th>
<th>Log proinsulin</th>
<th>Proinsulin/insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression coefficient (SE)</td>
<td>p</td>
</tr>
<tr>
<td>Family history of diabetes (yes/no)</td>
<td>0.125 (0.026)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.006 (0.003)</td>
<td>0.022</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.098 (0.003)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight (g)‡</td>
<td>−0.011 (0.003)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>0–hour 0.296 (0.014)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>2–hour 0.123 (0.006)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Univariate linear regression analyses.
† SE, standard error; BMI, body mass index.
‡ Per 100 g.

TABLE 4. Effects of family history of diabetes on proinsulin and proinsulin/immunoreactive insulin in relation to age, body mass index, birth weight, and glucose at 0 hours, Sweden, 1992–1994

<table>
<thead>
<tr>
<th>Variables included in the model*‡</th>
<th>Log proinsulin</th>
<th>Proinsulin/insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression coefficient (SE)</td>
<td>p</td>
</tr>
<tr>
<td>Family history of diabetes (yes/no)</td>
<td>0.125 (0.026)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Family history of diabetes (yes/no), age (years)</td>
<td>0.125 (0.026)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Family history of diabetes (yes/no), age (years), BMI (kg/m²)</td>
<td>0.049 (0.023)</td>
<td>0.036</td>
</tr>
<tr>
<td>Family history of diabetes (yes/no), age (years), BMI (kg/m²), birth weight (g)‡</td>
<td>0.044 (0.028)</td>
<td>0.120</td>
</tr>
<tr>
<td>Family history of diabetes (yes/no), age (years), BMI (kg/m²), birth weight (g), 2-hour glucose (mM)</td>
<td>0.006 (0.028)</td>
<td>0.837</td>
</tr>
</tbody>
</table>

* Multivariate linear regression analyses.
† SE, standard error; BMI, body mass index.
‡ Per 100 g.
Third, two other factors—obesity and low birth weight—were found to be associated with elevated proinsulins and P/IRI ratios. The possible confounding influence on these two factors could thus be corrected for.

Our study was limited to a rather homogeneous population of native Swedish men. Hence, our results cannot a priori be extrapolated to ethnically different populations. Some evidence indicates that there could be differences in levels of proinsulins and P/IRI ratios between Pima Indians and Caucasians (9). Further studies are needed to establish whether reported interpopulation differences are genetically determined.

It is relevant to note that our study did not investigate subjects with already known diabetes. The reasons for excluding such subjects were 1) an anticipated large proportion of such subjects with type 1 diabetes, and 2) the confounding effects of ongoing antidiabetic treatments in subjects with known diabetes. Not surprisingly, those subjects diagnosed with diabetes in this study had mild diabetes, which could be successfully treated by diet during the first year after discovery (data not shown). Whether levels of proinsulins and P/IRI ratios are also determined by other factors in more serious forms of diabetes has not been established.

The two groups in our study were closely age matched, but there was a difference in participation rates (higher for those with than for those without a family history of diabetes). This could potentially have led to some unwanted differences between the groups. However, it seems unlikely to us that any such putative difference would affect the interpretation of our findings.

A possible caveat pertains to the adequacy of bringing the plasma glucose level factor into the regression model. As reported above, the 2-hour plasma glucose levels during OGTT were positively associated with levels of proinsulins and P/IRI ratios not only at diabetic levels of glycemia but throughout the entire spectrum of glucose tolerances. An effect of glucose levels on levels of proinsulins and P/IRI ratios could be direct, that is, related to interaction with glucose or metabolites with the β cell. Alternatively—and, in our view, more likely—it could be indirect, being secondary to demands for insulin secretion in relation to insulin-releasing capacity. The importance of increased secretory demands in relation to capacity is supported by studies in hemipancreatectomized subjects (who, despite fasting normoglycemia, have elevated P/IRI ratios (29)), by animal studies in vivo (30), and by experiments in vitro with human islets (31).

Given that β-cell insufficiency is linked to fasting and/or elevated 2-hour plasma glucose levels, then including the glucose variable in a model along with family history will obscure a possible linkage of β-cell insufficiency (manifested as relative overstimulation) to family history of diabetes. Such a putative linkage would, however, only secondarily involve effects on levels of proinsulins and P/IRI ratios except if a genetic defect in proinsulin processing would result in β-cell insufficiency. Although the latter notion is theoretically possible, to our knowledge, there exists no evidence to this point. (The question would be testable in transgenic experiments.)

Our study has demonstrated a close association between obesity on one hand and elevated proinsulins and P/IRI ratios on the other. Although our findings on proinsulins are in line with other studies on obesity (17–20), our findings on P/IRI are not, since other studies find no increase in these ratios. As mentioned above, interpopulation differences may exist that could have a genetic background and that could be of importance for these discrepancies.

We also report an inverse relation between levels of proinsulins and P/IRI ratios on one hand and self-reported birth weight on the other. The effect of birth weight was significant, albeit small. Insulin resistance is associated not only with obesity but also with low birth weight (21). Insulin resistance is thus likely to be the underlying cause of effect of both of these factors. The importance of insulin resistance for the effect of BMI on levels of proinsulins and P/IRI ratios is further supported by the observation that the inclusion of fasting levels of IRI, a marker of insulin resistance, as an independent variable diminished the effect of BMI in the regression models.

It seems possible that glucocorticoids could participate in the influence of obesity and birth weight on proinsulins and P/IRI ratios. Hence, it was shown previously that dexamethasone-induced insulin resistance leads to elevated proinsulins and P/IRI ratios (32) and more recently that cortisol levels may be elevated both in subjects with obesity (33) and low birth weight (34).

The presently used regression models leave room for factors other than those presently analyzed. In particular, we do not have data to evaluate an influence of plasma levels of fatty acids, an influence that was recently documented in vitro in human pancreatic islets (31).

We have calculated proinsulins and P/IRI ratios by using an assay for proinsulin that comeasures major proinsulin intermediates and an assay for insulin that cross-reacts with proinsulins. Although these cross-reactivities were undesirable, they do not, in our view, affect the validity of our results, since 1) to our knowledge, proinsulin intermediates have not been shown to behave vastly differently from specific proinsulin in ratio analysis, and 2) having IRI rather than specific insulin in the denominator would not change the qualitative effect of any given factor on the ratio.

In conclusion, our analysis of a large, population-based sample enriched for family history of diabetes shows that glucose levels and insulin resistance markedly influence fasting levels of proinsulins and P/IRI ratios at all levels of glucose tolerance. Taking these factors into account, we do not find any evidence for a residual influence related to family history of diabetes on the processing of proinsulin.

ACKNOWLEDGMENTS

Supported by the Stockholm County Council, by the Swedish Medical Research Council (grant no. 72X-14070), and by the Swedish Diabetes Association.

The authors thank the many nurses and other staff members who carried out this investigation in practice. Special thanks are due to the coordinating efforts of Cecilia Ekehjelm.
REFERENCES


