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Fasting proinsulin and 2-h post-load glucose levels predict the conversion to NIDDM in subjects with impaired glucose tolerance: The Hoorn Study

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Summary The aims of the present study were to observe the natural history of impaired glucose tolerance and to identify predictors for development of non-insulin-dependent diabetes mellitus (NIDDM). A survey of glucose tolerance was conducted in subjects aged 50–74 years, randomly selected from the registry of the middle-sized town of Hoorn in the Netherlands. Based on the mean values of two oral glucose tolerance tests subjects were classified in categories of glucose tolerance according to the World Health Organization criteria. All subjects with impaired glucose tolerance ($n = 224$) were invited to participate in the present study, in which 70 % ($n = 158$) were subsequently enrolled. During follow-up subjects underwent a repeated paired oral glucose tolerance test. The mean follow-up time was 24 months (range 12–36 months). The cumulative incidence of NIDDM was 28.5 % (95 % confidence interval 15–42 %). Age, sex, and anthropometric and

metabolic characteristics at baseline were analysed simultaneously as potential predictors of conversion to NIDDM using multiple logistic regression. The initial 2-h post-load plasma glucose levels and the fasting proinsulin levels were significantly ($p < 0.05$) related to the incidence of NIDDM. Anthropometric characteristics, the 2-h post-load specific insulin levels and the fasting proinsulin/fasting insulin ratio were not related to the incidence of NIDDM. These results suggest that beta-cell dysfunction rather than insulin resistance plays the most important role in the future development of diabetes in a high-risk Caucasian population. [Diabetologia (1996) 39: 113–118]

Key words Impaired glucose tolerance, non-insulin-dependent diabetes mellitus, glucose tolerance test, proinsulin, specific insulin, beta-cell function, insulin resistance.

Subjects with impaired glucose tolerance (IGT) are at high risk of developing non-insulin-dependent diabetes mellitus (NIDDM). IGT is associated with genetic and environmental factors and is often characterized by insulin resistance. Various predictors for

the conversion from IGT to NIDDM have been reported [1–3]. In some studies high postprandial glucose values, high fasting C-peptide levels, high fasting and post-load insulin levels and a high waist-hip ratio were found to predict the development to diabetes mellitus in subjects with IGT, suggesting an important role for insulin resistance [1–11]. However, in other studies parameters of beta-cell dysfunction, such as a low insulin response to a glucose load, were found to be predictive for the conversion from IGT to diabetes [12–14]. A high fasting proinsulin level [15, 16] and a high proinsulin to insulin ratio [17, 18] have been used as measures of beta-cell dysfunction and the 2-h insulin level as a measure of insulin resistance [19, 20]. Predictors for the conversion from IGT to NIDDM have often been studied in

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Abbreviations: IGT, Impaired glucose tolerance; NIDDM, non-insulin-dependent diabetes mellitus; OGTT, oral glucose tolerance test; CI, confidence interval; W/H ratio, waist/hip ratio; BMI, body mass index; OR, odds ratio.

Fig. 1. Cumulative incidence of NIDDM according to four categories of the mean 2-h glucose level of the paired OGTT at baseline

Fig. 2. Cumulative incidence of NIDDM according to four categories of the fasting pro-insulin level at baseline

populations with a high diabetes prevalence but seldom in a homogeneous Caucasian population. In most studies the category of IGT has been defined on the basis of one oral glucose tolerance test (OGTT) only. The high variability of the OGTT may explain the wide range of the incidence rates reported for NIDDM in persons with IGT. In order to reduce the variability, subjects in the present study were classified on the basis of the mean value of two OGTTs.

The aims of our study were to assess the incidence of NIDDM and to analyse OGTT-derived measures of beta-cell function and insulin resistance as predictors for the development of NIDDM in a Caucasian population with IGT.

Subjects and methods

A cross-sectional survey of glucose tolerance was conducted from October 1989 to December 1991. Subjects (aged 50–75 years) were randomly selected from the registry of the mid-

dle-sized town of Hoorn. Based on the mean values of two OGTTs, which were performed 2–6 weeks apart, subjects were classified in categories of glucose tolerance according to the World Health Organization criteria, as described in detail previously [21]. Samples were taken at fasting (between 08.30 and 09.30 hours after a 10-h fast), and 120 min after a 75-g glucose load. Additional blood samples were taken for the measurements of HbA_{1c}, fasting and 2-h specific insulin, and fasting and 2-h proinsulin levels. Height, weight, waist and hip circumference were measured in a standardized manner. Of the subjects with IGT ($n = 224$) invited to participate in the present study 70% ($n = 158$) accepted. At an approximate 12-month interval subjects underwent a repeated paired OGTT, separated by 2 weeks. This paper reports the results of the first follow-up measurement. Conversion to diabetes was defined as a mean 2-h post-load plasma glucose level greater than 11.1 mmol/l. Body mass index (BMI) was calculated as weight (kg) divided by height (m²), waist to hip ratio (W/H ratio) according to the formula waist circumference divided by hip circumference.

Plasma glucose was measured with a glucose dehydrogenase method using the Granustest (Merck, Darmstadt, Germany). HbA_{1c} was measured with the high performance liquid chromatography method (HPLC-Biorad Diamat, Veenendaal, the Netherlands), normal range less than 6.1%. Plasma specific insulin level was measured using double-antibody radioimmunoassay (lot SP21; Linco Research, St. Louis, Mo., USA) in which proinsulin and 32, 33 split proinsulin cross-reacts by less than 0.2%. The inter-assay coefficient of variation was 6% (range 40–1000 pmol/l). The lower limit of sensitivity was 12 pmol/l. Plasma proinsulin level was measured by a double-antibody radioimmunoassay based on reagents from Dr. R. R. Bowsher (Lilly Laboratory for Clinical Research, Indianapolis, Ind., USA) as previously described [22]. In this assay des 31, 32-proinsulin cross-reacts by 63%. The inter-assay coefficient of variation was 6% at levels of 100 pmol/l and increased to 15% at lower levels. The lower limit of detection was 3 pmol/l.

Statistical analysis

For the statistical analysis a value under the lower limit of detection was set at 3 pmol/l. For 28 subjects, the proinsulin value at baseline was missing, due to missing blood samples. All the characteristics of the insulin and proinsulin assays were derived in our laboratory at the Department of Endocrinology.

Due to the variable follow-up time, both the incidence density, the number of cases divided by the observed time at risk, and the cumulative incidence for NIDDM were calculated. The 95% confidence interval (95% CI) was calculated using the method described by Miettinen [23]. The baseline characteristics of non-converters and converters to NIDDM are reported. For the normally distributed variables means and 95% CI are reported. The variables of glucose metabolism, which showed a skewed distribution, are depicted with their medians and the corresponding 25th and 75th percentiles. In order to assess whether the risk for conversion to NIDDM showed a trend with some determinants such as the glycaemic indices and other potential determinants, we first constructed four-point scales by dividing the original continuous scales into four equal ranges (Figs. 1 and 2). The chi-square test for linear trend for the conversion to NIDDM was then applied. To study the relative contribution to the conversion to NIDDM of the different variables, a forward stepwise multiple logistic regression was used with SPSSPC + 5.0 software. Age and sex were entered first in the regression equation as potential confounders. Subsequently, the other independent vari-

Table 1. Baseline characteristics of non-converters and converters to NIDDM

	Non-converters <i>n</i> = 113	Converters to NIDDM <i>n</i> = 45	<i>p</i> -value
	<i>Mean (95 % CI)</i>	<i>Mean (95 % CI)</i>	
Age (years)	63.7 (56.4–71.1)	65.3 (58.2–72.4)	0.15
Percentage male (%)	43	47	
Body mass index (kg/m ²)	27.6 (24.3–31)	28.4 (24.4–32.3)	0.79
Waist-hip ratio			
male	0.95 (0.88–1.02)	0.98 (0.92–1.04)	0.29
female	0.89 (0.81–0.97)	0.92 (0.84–1.0)	0.61
	<i>Median (25th–75th perc)</i>	<i>Median (25th–75th perc)</i>	
HbA _{1c} (%)	5.5 (5.2–5.9)	5.7 (5.3–6.0)	0.08
Fasting plasma glucose (mmol/l)	5.9 (5.6–6.4)	6.1 (5.6–6.6)	0.02
2-h Plasma glucose (mmol/l)	8.6 (8.1–9.4)	9.3 (8.6–9.9)	< 0.01
Fasting specific insulin (pmol/l)	89.4 (70.7–125.8)	98.7 (73.1–141.6)	0.16
2-h Specific insulin (pmol/l)	546.1 (390.5–906.4)	632.5 (424.9–1044.8)	0.21
Fasting proinsulin (pmol/l)	12.1 (5.8–20.0)	17.3 (10.9–25.2)	0.01
2-h Proinsulin (pmol/l)	74.9 (58.6–109.5)	83.5 (51.1–134.3)	0.25
Proinsulin/insulin ratio	0.13 (0.07–0.22)	0.17 (0.11–0.23)	0.12

P values are chi-square for linear trend. Perc, Percentiles

Table 2. Multiple logistic regression analysis with conversion to NIDDM as dependent variable

Variable	Odds ratio	95 % Confidence interval	<i>p</i> -value
<i>Model 1</i>			
Age (1-year difference)	1.04	0.97–1.11	0.29
Sex (female, male)	1.02	0.39–2.68	0.97
2-h Plasma glucose (1 mmol/l difference)	2.11	1.21–3.7	< 0.01
Fasting proinsulin (10 pmol difference)	1.57	1.05–2.34	0.03
Variables not in equation: BMI, W/H ratio, HbA _{1c} , fasting and 2-h glucose level, fasting and 2-h specific insulin level, fasting and 2-h proinsulin level, and the product-term of sex and W/H ratio			
<i>Model 2</i>			
Age (1-year difference)	1.04	0.97–1.11	0.23
Sex (female, male)	1.07	0.42–2.73	0.88
Body mass index (1 g/m ² difference)	1.01	0.89–1.14	0.85
Beta-cell function parameter: fasting proinsulin (10 pmol difference)	1.67	1.1–2.51	< 0.02
Insulin resistance parameter: 2-h Spec insulin (10 pmol difference)	1.00	0.99–1.01	0.89
<i>Model 3</i>			
Age (1-year difference)	1.02	0.93–1.12	0.71
Sex (female, male)	1.02	0.23–2.91	0.76
2-h Plasma glucose (1 mmol/l difference)	2.18	1.05–4.53	0.04
Fasting proinsulin (10 pmol difference)	1.94	1.09–3.47	0.02
Variables not in equation: BMI, W/H ratio, HbA _{1c} , fasting and 2-h glucose level, fasting and 2-h specific insulin level, fasting and 2-h proinsulin level, and the product-term of sex and W/H ratio			

ables with the best fit were entered stepwise into the equation. A *p*-value of 0.05 was used as the criterion for entry at each step. A backward procedure was also used, but both the forward and backward methods led to the same model.

Interaction between sex and W/H ratio was tested using a product-term. The distribution of proinsulin and glucose values, which are independent variates in the regression, were skewed. To check whether a logarithmic transformation would improve the fit of the model, the log likelihood of the predominant measure of goodness of fit was also analysed after transformation. It appeared that after transformation the goodness of fit actually decreased. To analyse the contribution for the conversion to NIDDM of the fasting proinsulin level (as a possible marker of beta-cell dysfunction), and the BMI, fasting and 2-h specific insulin (as potential markers of insulin resis-

tance) these variables were entered in a second logistic regression model and adjusted for age and sex.

A stepwise procedure of logistic regression analysis was also executed to study the relative contribution to the conversion to NIDDM in subjects who showed NIDDM at both follow-up OGTTs. Subjects who scored once for NIDDM and once for IGT were then excluded from this analysis. Residual plots were used to check the assumptions of all the regression models. For the various described logistic regression analyses, the magnitude of the relative risk is presented as odds ratios with 95 % CI and *p*-values.

Results

The mean follow-up time was 24 months (range 12–36 months). The incidence density of NIDDM was $13.8\% \cdot \text{year}^{-1}$ (95% CI 3.5–24); the cumulative incidence was 28.5% (95% CI 15–42). At baseline seven subjects had, at one of the two OGTTs, a 2-h plasma glucose level greater than 11.1 mmol/l; four of these subjects developed NIDDM during follow-up. Of the 33 subjects with a 2-h plasma glucose level less than 7.8 mmol/l, at one of the two OGTTs, seven developed NIDDM. Table 1 presents the various baseline characteristics for the non-converters and for those who converted to NIDDM, with the *p*-values of the chi-square test for linear trend. In this univariate analysis the fasting, 2-h plasma glucose and the fasting proinsulin levels were significantly associated with the conversion to NIDDM. At baseline 12% (*n* = 19) of subjects had HbA_{1c} levels greater than 6.1%, of whom 52.6% progressed to NIDDM.

Figure 1 shows the cumulative incidence of NIDDM according to the initial 2-h plasma glucose level. The proportion of subjects converting to NIDDM during follow-up increased with ascending baseline mean 2-h plasma glucose levels.

Figure 2 shows the cumulative incidence of NIDDM according to the fasting proinsulin level at baseline, indicating an increasing risk for conversion at higher baseline fasting proinsulin levels.

Table 2 presents three logistic regression models with the conversion to NIDDM as dependent variable. The first model presents the best fit after entering stepwise the independent variables into the regression equation, with age and sex entered first as potential confounders. Other independent variables were BMI, W/H ratio, HbA_{1c}, fasting and 2-h glucose level, fasting and 2-h specific insulin level, fasting and 2-h proinsulin level, and the product-term of sex and W/H ratio. Significant associations were found for the 2-h plasma glucose and the fasting proinsulin levels. The logistic regression model with the HbA_{1c} level as the independent variable instead of the fasting and the 2-h glucose level, demonstrated a significant association for HbA_{1c} with the incidence of NIDDM (data not shown). In the second model markers for beta-cell dysfunction and insulin resistance are entered already adjusted for age and sex. In the third model the conversion to NIDDM is defined as a 2-h glucose value greater than 11.1 mmol/l at both follow-up OGTTs (*n* = 28). The independent variables were entered stepwise in the regression equation. As in the first model significant associations were found for the 2-h plasma glucose and the fasting proinsulin level.

Discussion

The results of the present study indicate that the incidence of NIDDM in Caucasian subjects with stable impaired glucose tolerance is higher than has been previously reported. When interpreting the cumulative incidence, the variable follow-up time (ranging from 12–36 months) has to be taken into account. The cumulative incidence of NIDDM was 28.5% over an average period of 2 years in our study, which is clearly higher than in similar studies among other Caucasian populations [24–26]. The major reason is probably the use of more stringent criteria for both IGT and NIDDM; for qualification the mean value of two OGTTs was used. If selection of IGT is based on a single OGTT, a substantial proportion falls outside the IGT range on repeated testing [27–29]. Our rate of conversion was similar to studies in impaired glucose tolerant South African Indians, Micronesian Nauruans and Pima Indians [30–33]. However, it should be noted that in those studies subjects were qualified on the basis of one OGTT only.

As in other studies, the initial level of the 2-h post-load plasma glucose level predicted the conversion to NIDDM in persons with IGT [24–26, 34, 35]. In our study also a high initial HbA_{1c} level, a long-term indicator of glycaemia, was found to be related to the conversion to diabetes. In Pima Indians a similar association was reported [36].

Increased secretion of proinsulin has been associated with beta-cell dysfunction, either in absolute terms or as related to the secretion of insulin [15–18, 37–42]. Hyperproinsulinaemia is thought to be a pancreatic beta-cell defect that is augmented by the increased demand placed on the beta-cell by hyperglycaemia [16]. It has also been suggested that increased proinsulin levels in subjects with glucose intolerance are at least partly a result of a defective feedback inhibition of proinsulin secretion by insulin [43]. The fasting proinsulin level at baseline, adjusted for age, sex, and the 2-h plasma glucose level, showed a significant association with the conversion to NIDDM. However, the assay used in this study is unable to differentiate between intact proinsulin and des 31, 32 proinsulin. The inhibition of the conversion from des 31–32 proinsulin by hyperglycaemia, could to some extent overestimate the contribution of our measure of beta-cell dysfunction. Despite the absence of an association between conversion and insulin levels, the proportional proinsulin-to-insulin level was not found to be related to conversion to NIDDM (data not shown). This is an apparent inconsistency in the data. On the other hand the pitfalls of using a ratio of two biological parameters must be considered, noting the wider range and skewed distribution of the insulin levels relative to those of proinsulin. Moreover, the assumption of an increasing linear association between insulin and proinsulin has to be made which is clearly not the case.

The absence of an association for the fasting and post-load insulin level with the conversion to NIDDM, both proposed as measures of insulin sensitivity [19, 20, 44–46], at least indirectly supports our interpretation of the relation between fasting proinsulin and 2-h glucose values. We also failed to demonstrate an association of measures of obesity or fat distribution, i.e. the BMI and W/H ratio, with conversion to NIDDM. This agrees with the results of the Bedford and Whitehall studies [24, 25]. In these studies only subjects with IGT were followed longitudinally. However, in various other prospective population-based studies, also including subjects with normal glucose tolerance, body weight and central obesity were associated with the incidence of diabetes [5, 6, 34, 47–49]. In euglycaemic hyperinsulinaemic clamp studies, the development of diabetes in persons with normal glucose tolerance, was predominantly associated with insulin resistance [50–52]. Saad et al. [14] hypothesised a two-step model for the development of diabetes. The first step, the transition from normal to impaired glucose tolerance, depends mainly on the presence of insulin resistance. The second step, worsening from IGT to diabetes, although accompanied by some further worsening of insulin resistance, appears to be primarily dependent on the development of beta-cell dysfunction. The results of our study agree with this hypothesis.

We conclude that our study has demonstrated a high incidence of diabetes in a Caucasian population with IGT, selected on the basis of the mean values of two OGTTs. Furthermore, the results of our study suggest that beta-cell dysfunction, rather than insulin resistance, plays a more important role in the future development of NIDDM in a Caucasian IGT population.

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