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Cardiovascular Risk Factors Prior to the Development of Non-Insulin-Dependent Diabetes Mellitus in Persons with Impaired Glucose Tolerance: The Hoorn Study

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ABSTRACT. The aim of the study was to analyze cardiovascular risk factors as predictors for developing non-insulin-dependent diabetes mellitus (NIDDM) in people with impaired glucose tolerance. A cross-sectional survey of glucose tolerance was conducted in people, aged 50–74, who were randomly selected from the registry of the middle-sized town Hoorn (The Netherlands). Based on the mean values of two oral glucose tolerance tests, people were classified in glucose tolerance categories according to the WHO criteria. The mean follow-up time was 36 months (range 13–55 months). The cumulative incidence of NIDDM was 34% (95% CI 16.9–45.1). In multiple logistic regression analysis, cardiovascular risk factors at baseline did not predict the conversion from impaired glucose tolerance to NIDDM, in contrast with the two-hour plasma glucose level (odds ratio 3.56, $p < 0.001$) and the fasting proinsulin level, as one of the determinants of beta-cell dysfunction (Odds ratio 2.1, $p < 0.05$). The baseline HDL-cholesterol level, one of the components of the insulin resistance syndrome, was associated with the conversion from impaired glucose tolerance to normal glucose tolerance (Odds ratio 1.58, $p < 0.05$). The results of our study seem to support the hypothesis that conversion from impaired glucose tolerance to normal glucose tolerance depends on insulin resistance and the development of NIDDM from impaired glucose tolerance depends on beta-cell dysfunction. J CLIN EPIDEMIOL 50;9:1003–1009, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. Cardiovascular risk factors, impaired glucose tolerance, insulin resistance, non-insulin-dependent diabetes mellitus, proinsulin

INTRODUCTION

Since the World Health Organization (WHO) defined impaired glucose tolerance [1–3], there have been several cross-sectional and prospective studies published on the prevalence of cardiovascular risk factors among people with impaired glucose tolerance and with newly diagnosed non-insulin-dependent diabetes mellitus (NIDDM) [4–12]. Both in cross-sectional studies and in prospective studies increasing levels of cardiovascular risk factors with decreasing glucose tolerance have been found. Furthermore, people with impaired glucose tolerance turn out to be at substantial risk of developing cardio- and cerebrovascular diseases: the mortality due to these causes is approximately 2-fold compared with people with normal glucose tolerance [13–15]. The joint presence of unfavorable levels of the various cardiovascular risk factors, including hypertension, high triglyceride levels, and low HDL-cholesterol levels, is considered to reflect insulin resistance. This clustering has been labeled as Syndrome X or insulin resistance syndrome [16–21].

Saad *et al.* [22] hypothesized a two-step model for the development of diabetes in Pima Indians. The first step, the transition from normal to impaired glucose tolerance, would depend mainly on the presence of insulin resistance. The second step, worsening from impaired glucose tolerance to diabetes, although accompanied by some further worsening of insulin resistance, is assumed to be primarily dependent on the development of beta-cell dysfunction. It is unclear if this is also the case in a Caucasian population. We previously reported the associations of the measurements of the glucose metabolism, including insulin and proinsulin levels, with the conversion to NIDDM in people with impaired glucose tolerance after 15 months of follow-up [23]. The

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fasting proinsulin level predicted the conversion to NIDDM suggesting that beta-cell dysfunction, rather than insulin resistance, plays a more important role in the future development of NIDDM in an impaired glucose intolerant Caucasian population. In this study the cardiovascular risk factors, as major components of the insulin resistance syndrome, are analyzed as potential predictors of the conversion to NIDDM and normal glucose tolerance. To study a well-defined impaired glucose tolerant category, people were classified on the basis of the mean value of two oral glucose tolerance tests. Previous studies, which reported the incidence of NIDDM in Caucasian populations, the category of impaired glucose tolerance has been defined on the basis of one oral glucose tolerance test only [9–11]. Furthermore, in those studies specific insulin and proinsulin were not measured and not all the determinants of the lipid metabolism were analyzed.

The aims of the present study were to assess the incidence of NIDDM and to analyze the various cardiovascular risk factors at baseline as predictors of the conversion to NIDDM and to normal glucose tolerance.

SUBJECTS AND METHODS

Subjects

From October 1989 to December 1991 a cross-sectional survey of glucose tolerance was conducted. People aged 50–74 years were randomly selected from the computerized registry of the mid-sized town of Hoorn in The Netherlands, which has approximately 59,000 (mixed rural and urban) inhabitants.

Oral Glucose Tolerance Tests

Based on the mean values of two paired oral glucose tolerance tests, which were performed 2–6 weeks apart, persons were classified in categories of glucose tolerance according to the WHO criteria, as described in detail previously [24]. Samples were taken at fasting (between 8.30 and 9.30 am after a 10-hour fast), and 120 minutes after a 75-gram glucose load. Additional blood samples were taken for the measurements of fasting and 2-hour specific insulin, and fasting and 2-hour proinsulin levels. Height, weight, and waist and hip circumference were measured in a standardized way.

Follow-Up Visits

Of the persons with impaired glucose tolerance (n = 224) invited to participate in the present study 70% (n = 158) accepted. The baseline characteristics from non-participants in the study did not differ from the participating people. In a non-parametric Mann-Whitney test no statistically significant difference was found. At 13–18-month intervals, people underwent a repeated oral glucose tolerance test, separated by two weeks. This paper reports the results of two follow-up measurements. People with NIDDM according to the WHO criteria were referred to the general practitioner. In the case of conversion to NIDDM at the first follow-up visit only the results of the first follow-up visit were analyzed. All persons with no conversion to NIDDM participated in the second follow-up visit.

Baseline Measurements

Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters squared), waist to hip ratio (W/H ratio) were taken, according to a standardized procedure [25], as the horizontal circumference halfway between the lower rib margin and the iliac crest, and the point of the maximum circumference over the buttocks, respectively. The W/H ratio was defined as waist circumference divided by hip circumference. Blood pressure was measured on the right arm of each seated person, after at least five minutes rest, with a random zero mercury sphygmomanometer (Hawksley-Gelman). The average of two readings of systolic and diastolic blood pressure (Korotkoff V) to the nearest even digit was recorded. Fasting serum cholesterol and HDL-cholesterol were measured with the enzymatic calorimetric method (CHOD-PAP, Boehringer Mannheim), fasting serum triglyceride with the enzymatic calorimetric method (CPO-PAP, Boehringer Mannheim). Fasting and 2-hour plasma glucose levels, 2–6 weeks apart, were measured with a glucose dehydrogenase method using the Granu-test (Merck). The inter-assay coefficient of variation was 1.4%. Fasting and 2-hr plasma specific insulin levels were measured using double-antibody radio-immunoassay (lot SP21, Linco Research, St Louis, MO) in which proinsulin and 32/33 splitproinsulin cross-reacts by <0.2%. The inter-assay coefficient of variation was 6% in the range of 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, IN) as previously described [24]. 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. All the characteristics of the assays were derived at our laboratory of the Department of Endocrinology.

Data Analyses

Based on the mean values of two paired oral glucose tolerance tests, which were performed two weeks apart, persons were classified in categories of glucose tolerance according to the WHO criteria (mean 2-hr post-load plasma glucose level greater than 11.1 mmol/l). Because of the variable follow-up time, both the incidence density—the number of

cases devided by the observed time at risk—and the cumulative incidence for the conversion to NIDDM were calculated. The 95% confidence interval was calculated using the method described by Miettinen [26]. The baseline characteristics of non-converters, converters to normal glucose tolerance, and converters to NIDDM are depicted with their medians and the corresponding 20th and 80th percentiles. The plasma glucose values and the specific insulin values are the average of two measurements of the paired oral glucose tolerance test. For analyzing the crude relative risk for conversion to NIDDM the cardiovascular risk factors were dichotomized [27,28]. As cut-off point for hypercholesterolaemia a level > 6.2 was used and for hypertriglyceridemia level > 2.2 mmol/l. Because mean HDL-cholesterol levels are approximately 0.3 mmol/l higher in women, different cut-off points were chosen for the definition of abnormal HDL-cholesterol levels in men and women: < 0.9 and < 1.2 mmol/l, respectively. Hypertension was defined by diastolic BP > 95 and/or systolic BP > 160 mmHg, or current use of antihypertensive medication [28]. The chi-squared test was used for evaluating the null hypothesis that conversion to NIDDM and a high cardiovascular risk factor were independent. To analyze potential predictors of the incidence of conversion to NIDDM on the one hand, and conversion to normal glucose tolerance on the other hand, two backward stepwise multiple logistic regression analyses were used with age, sex, and months of follow-up as potential confouders and the other independent variables (the cardiovascular risk factors at baseline, the specific insulin levels and anthropometric measurements) entered stepwise into the equation. As reference category non-converters and converters in the opposite direction was used. A *p*-value of 0.05 was used as the criterion for entry at each step. Different interaction terms were entered, as were the ratio between triglyceride level and HDL-cholesterol level. After the backward procedure procedure, a forward procedure was used. All statistical analyses were performed with the SPSS-PC 5.0 statistical package [29].

RESULTS

The mean follow-up time was 36 months (range 13–55 months). The cumulative incidence of NIDDM was 34% (95% CI 16.9–45.1). The incidence density was 0.12/year (95% CI 0.02–0.22). Table 1 presents the differences between participants and non-participants. In a non-parametrische Wilcoxon test none of the differences between participants and non-participants were significant.

Table 2 presents the baseline characteristics for the non-converters, converters to normal glucose tolerance or to NIDDM and for all people.

Figure 1 presents the prevalence rates of high cardiovascular risk factors at baseline in the three categories of glucose tolerance at the end of follow-up. As can be seen from the figure about 70% of the subjects with impaired glucose tolerance have a high cholesterol level at baseline, 28.5% a high triglyceride level, about 30% a low HDL-cholesterol and finally about 45% a high blood pressure at baseline.

Table 3 presents the unadjusted relative risk of a high cardiovascular risk factors dichotomized as described in the method paragraph, for the incidence of conversion to NIDDM and the results of chi-squared analyses. No statistically significant associations of the cardiovascular risk factors with the incidence of conversion to NIDDM were present. In a separate model, where the ratio triglyceride/HDL-cholesterol was used instead of triglyceride and HDL cholesterol, no significant associations were found with the incidence of NIDDM.

Table 4 presents the result of a multiple logistic regression analyses with the conversion to NIDDM as the dependent

TABLE 1. Differences between participants and non-participants in the study

	Participants (percentile)			Non-participants (percentile)		
	50	25	75	50	25	75
Sex						
Female	58.5%			58.8%		
Male	41.5%			41.2%		
Age	65.4	59.7	70.3	66.6	60	70.7
Body Mass Index	27.7	25.4	30	27.4	25.3	30
Waist/Hip ratio	0.93	0.85	0.99	0.93	0.87	0.97
Fasting plasma glucose (mmol/l)	6	5.6	6.4	5.7	5.4	6.1
2-hour plasma glucose (mmol/l)	8.9	8.2	9.6	8	7.4	9.7
Fasting spec. insulin (pmol/l)	95.7	72.2	125.6	82.3	62.8	136.6
2-hour spec. insulin (pmol/l)	589.8	408.8	887.3	518.6	326.6	876.6
Cholesterol (mmol/l)	6.9	6	7.4	6.5	5.8	7.3
HDL cholesterol (mmol/l)	1.2	0.99	1.46	1.2	1.01	1.43
Triglycerides (mmol/l)	1.7	1.3	2.4	1.6	1.2	2.2

TABLE 2. Baseline characteristics of non-converters and converters to normal glucose tolerance, non-insulin diabetes mellitus (NIDDM), and the total cohort

	Non-converters $n = 67$	Converters to NIDDM $n = 56$	Converters to NGT $n = 35$	Total $n = 158$
Age (year)	63.9 (57.1–71.6)[a]	66.4 (59–72.8)	59.8 (53.9–69.7)	63.9 (57.1–71.3)
Months follow-up	36.5 (31–45.5)	32 (21–41.5)	39 (34–44)	36 (17–45)
Percentage male (%)	46.3	49.1	33.3	
Body mass index (kg/m^2)	27.4 (25.4–30.4)	27.5 (25.2–31.4)	26.6 (24.4–29.9)	27.3 (24.9–30.4)
Waist/hip ratio				
Male	0.96 (0.94–1.0)	0.98 (0.9–1.01)	0.91 (0.84–1.01)	0.97 (0.90–1.01)
Female	0.89 (0.82–0.98)	0.92 (0.84–1.0)	0.88 (0.82–0.95)	0.89 (0.83–0.98)
Blood pressure				
Systolic	140 (128–158)	138 (124–158)	139 (130–159)	140 (126–158)
Diastolic	86 (78–92)	82 (76–90)	83 (78–92)	84 (78–90)
HbA_{1c} (%)	5.5 (5.1–5.9)	5.7 (5.4–6.2)	5.4 (5.0–6)	5.6 (5.2–6)
Fasting plasma glucose (mmol/l)	5.5 (5.1–5.9)	6.2 (5.7–6.8)	5.9 (5.4–6.3)	5.9 (5.5–6.6)
2-hour plasma glucose (mmol/l)	8.6 (8.2–9.1)	9.4 (8.5–10.3)	8.7 (8.1–9.7)	8.9 (8.2–9.9)
Fasting specific insulin (pmol/l)	87.4 (59.6–128.4)	98.8 (69.1–155.7)	91.6 (72.2–138.3)	93.9 (65.3–137.9)
2-hour specific insulin (pmol/l)	551 (417.5–1043.8)	620.4 (361.2–1235.1)	538.8 (344.4–1017)	589.6 (365.4–1068)
Cholesterol (mmol/l)	7.0 (5.8–8.1)	6.9 (5.8–7.8)	6.9 (6.0–7.3)	6.9 (5.9–7.9)
Triglycerides (mmol/l)	1.7 (1.2–2.4)	1.9 (1.3–3.0)	1.5 (1.1–2.5)	1.7 (1.2–2.7)
HDL-cholesterol (mmol/l)				
Male	1.0 (0.89–1.2)	1.09 (0.88–1.49)	1.21 (0.9–1.42)	1.01 (0.89–1.35)
Female	1.26 (1.05–1.58)	1.14 (0.93–1.49)	1.42 (1.15–1.61)	1.28 (1.02–1.57)

[a]Median (20–80 percentiles).

variable. The model presents the best fit after entering stepwise the independent variables into the regression equation, with age, sex and months of follow-up entered first as potential confounders. Other independent variables were BMI, W/H ratio, HbA_{1c}, 2-hr glucose level, fasting and 2-hr specific insulin level, and fasting and 2-hr proinsulin level. About the same significant associations were found for the 2-hr glucose level and fasting proinsulin level as earlier described after 24 months of follow-up [23].

Table 5 presents the results of two multiple logistic regression analyses. In the first model the conversion of NIDDM is used as the dependent variable. In the second model the conversion to normal glucose tolerance is used as the dependent variable. Both models present the best fit after entering

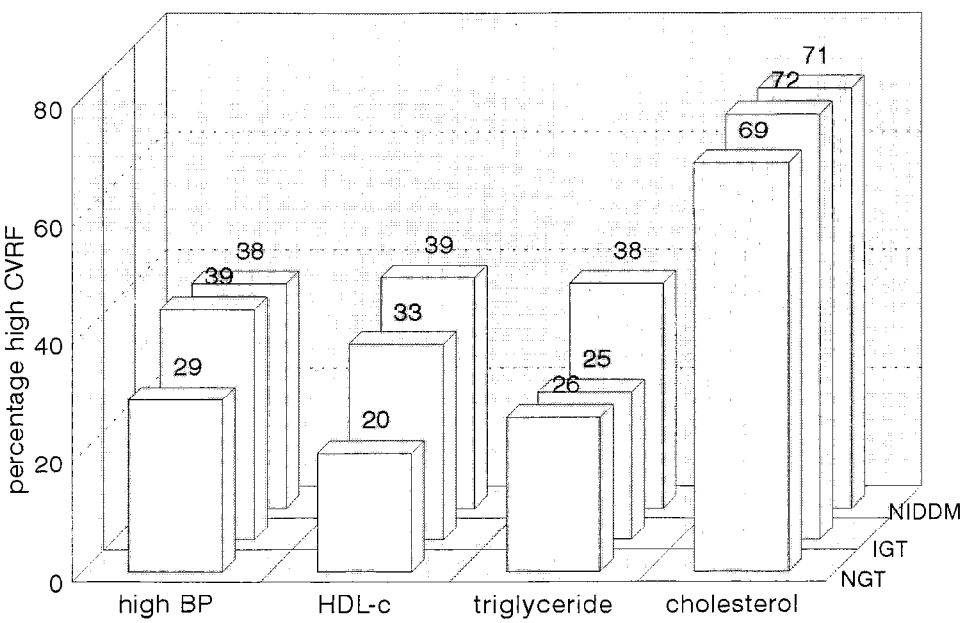

FIGURE 1. Percentage of a high level of each cardiovascular risk factor at baseline in the three categories of glucose tolerance category at follow-up.

TABLE 3. The relative risk conferred by the presence of catgorised cardiovascular risk factor for the incidence of NIDDM

	Relative risk NIDDM (95% CI)	Chi square	*p*-value
Cholesterol > 6.1 mmol/l	1.03 (0.64–1.64)	0.01	0.91
Low HDL-cholesterol (mmol/l)			
Female	1.60 (0.86–2.98)	2.2	0.14
Male	1.29 (0.71–2.33)	0.63	0.43
High triglyceride (mmol/l)	1.42 (0.93–2.26)	2.5	0.11
Hypertension (WHO-criterion)	1.06 (0.69–1.64)	0.78	0.78

TABLE 4. Multiple logistic regression analysis with conversion to NIDDM as dependent variable

Variable	Odds ratio	95% confidence interval	*p*-Value
Age (1-year difference)	1.06	0.36–3.08	0.29
Sex (female/male)	0.48	0.18–1.31	0.97
2-hour plasma glucose (1 mmol/l difference)	3.56	1.84–6.89	<0.01
Fasting proinsulin (10 pmol difference)	2.1	1.02–4.3	0.04

Variables not in equation: BMI, W/H ratio, HbA_{1c}, fasting, and 2-hour glucose level, fasting and 2-hour specific insulin level, fasting and 2-hour proinsulin level, hypertension (WHO criterion), and the product-term of sex and W/H ratio.

TABLE 5. Multiple logistic regression analysis with conversion to NIDDM as dependent variable in the first model and conversion to NGT as the dependent variable in the second model

Variable	Odds ratio	95% confidence interval	*p*-Value
Model 1: dependent variable: conversion to NIDDM			
Variables in the equation			
Age (1 year difference)	1.05	0.99–1.11	0.07
Sex (f/m)	0.49	0.23–1.09	0.08
Months follow-up (1 month)	0.94	0.90–0.98	0.04
Variables not in the equation			
Triglyceride, HDL-cholesterol, fasting and 2-hour specific insulin level, hypertension (WHO criterion), W/H ratio			
Model 2: dependent variable: conversion to NGT			
Variables in the equation			
Age (1 year difference)	0.94	0.88–0.99	0.04
Sex (f/m)	1.95	0.71–5.32	0.2
Months follow-up (1 month)	1.03	0.98–1.08	0.19
HDL-cholesterol (1 mmol/l difference)	1.58	1.17–20.4	0.03
Variables not in the equation			
Triglyceride, fasting and 2-hour specific insulin level, hypertension (WHO criterion), W/H ratio			

stepwise the independent variables into the regression equation, with age, sex, and months of follow-up entered first as potential confounders. Other independent variables were W/H ratio, hypertension (WHO criterion), the fasting insulin level, the triglyceride level, the cholesterol level, and the HDL-cholesterol level as the independent variables. Transformation of the skewed distributed variables and interaction terms did not improve the fit of the models. No statistically significant association of the various cardiovascular risk factors with the incidence of NIDDM was found. In both the stepwise foreward analyses and the stepwise backward analyses, a statistically significant association was found between the HDL-cholesterol level and the conversion to normal glucose tolerance.

DISCUSSION

The cumulative incidence of NIDDM over an average period of three years was 34%, which is clearly higher than in similar studies among other Caucasian populations [10–12]. The major reason for this might be the use of more stringent and precise criteria for both impaired glucose tolerance and NIDDM, as the mean value of two oral glucose tolerance tests was used for qualification. It has been proposed that low HDL cholesterol and high serum triglyceride levels may be due to reduced insulin sensitivity. In combination with hypertension, hyperinsulinaemia, and glucose intolerance, it constitutes Reaven's "Syndrome X" or the insulin resistance syndrome [15–20]. In the two step model hypothesis for the development of NIDDM, the first step from normal glucose tolerance to impaired glucose tolerance would be predominantly determined by insulin resistance. Manifestations of insulin resistance are high fasting and post-load insulin levels, high triglycerides and low HDL-cholesterol levels, and a high W/H ratio [30–32]. In the cross-sectional part of the Hoorn Study, as in other studies, high fasting and high post-load insulinemia identified subjects with dyslipidemia and hypertension [33–35]; moreover, subjects with impaired glucose tolerance and NIDDM have a 2–4 times higher risk for dyslipidaemia and hypertension than subjects with normal glucose tolerance. The second step, the transition from impaired glucose tolerance to NIDDM would be predominantly determined by beta-cell dysfunction accompanied with some further worsening of insulin resistance. A high fasting proinsulin level [36,37] and a high proinsulin to insulin ratio [38,39] have been used as measures of beta-cell dysfunction. Recent studies have reported that the insulin secretory capacity is impaired in impaired glucose tolerance [40–45]. As also reported after one follow-up measurement, the fasting proinsulin level, apart from the 2-hr plasma glucose level, predicts the conversion to NIDDM in people with impaired glucose tolerance, suggesting beta-cell dysfunction [22]. The HDL-cholesterol, the triglyceride levels and the insulin levels, as components of the insulin resistance syndrome, do not predict the conversion from impaired glucose tolerance to NIDDM. However high HDL cholesterol levels predict the conversion from impaired glucose tolerance to normal glucose tolerance, suggesting a role for insulin sensitivity, here some study limitations have to be mentioned. The interpretation of the fasting proinsulin level as a reflection of beta-cell function is a potential problem. The assay used in our study was not able to differentiate between intact proinsulin and des 31–32 proinsulin. The inhibition of the conversion from des 31–32 proinsulin to insulin by hyperglycemia, may therefore have overestimated the contribution of beta-cell dysfunction as reflected by the high proinsulin levels found in our study. The fact that high HDL cholesterol predicts the conversion from impaired glucose tolerance to normal glucose tolerance is probably a reflection of a more healthy lifestyle behavior; moreover, we did not study the conversion from normal glucose tolerance to impaired glucose tolerance. For that reason we have planned a prospective study in subjects with normal glucose tolerance to study the relative contribution of beta-cell dysfunction for the conversion from normal glucose tolerance to impaired glucose tolerance. In summary, in Caucasian people with well-defined impaired glucose tolerance, the cumulative incidence of NIDDM is high and our results are in line with the hypothesis of the two-step model of the development of NIDDM.

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