Proinsulin Concentration Is an Independent Predictor of All-Cause and Cardiovascular Mortality

An 11-year follow-up of the Hoorn Study

MARJAN ALSSEMA, MSC[1]
JACQUELINE M. DEKKER, PHD[1]
GIEL NIJPELS, MD, PHD[1,2]
COEN D.A. STEHOUWER, MD, PHD[1,3]
LEX M. BOUTER, PHD[1]
ROBERT J. HEINE, MD, PHD[1,4]

OBJECTIVE — High proinsulin concentration may be a better predictor for cardiovascular disease (CVD) mortality than insulin concentration. Previous observations may have been confounded by glucose tolerance status or lack of precision because of high intraindividual variability. We investigated the longitudinal relation of means of duplicate measurements of insulin and proinsulin with all-cause and CVD mortality in a population-based cohort taking glucose tolerance status into account.

RESEARCH DESIGN AND METHODS — Fasting and post–75-g glucose-load (2-h) glucose, insulin, and proinsulin values were determined in duplicate on separate days in 277 participants with normal glucose metabolism, 208 participants with impaired glucose metabolism, and 119 newly detected patients with type 2 diabetes of the Hoorn Study. Insulin resistance and β-cell function were estimated by homeostasis model assessment (HOMA-IR and HOMA-B, respectively), and the fasting proinsulin-to-insulin ratio was calculated. Subjects were followed with respect to mortality until January 2003.

RESULTS — Fasting proinsulin levels were significantly associated with all-cause and CVD mortality. The hazard ratios (HRs) per increase in interquartile range adjusted for age and sex were 1.21 (95% CI 1.04–1.42) for all-cause mortality and 1.33 (1.06–1.66) for CVD mortality. Adjustment for glucose tolerance status and HOMA-IR did not substantially change the associations.

CONCLUSIONS — Fasting proinsulin was associated with all-cause and CVD mortality, independent of glucose tolerance status and insulin resistance and largely independent of other CVD risk factors. Proinsulin might play a role in the relationship between insulin resistance and CVD.

Diabetes Care 28:860–865, 2005

Hyperinsulinemia is a marker of insulin resistance and was associated with all-cause mortality (1) and cardiovascular disease (CVD) mortality in prospective studies in the general population (2–6). The association between postload insulin and CVD mortality was found to be weaker than the association between fasting insulin and CVD mortality (2,7,8).

A number of population-based studies showed that proinsulin, the precursor of insulin, is a better predictor of coronary heart disease than insulin (9–12). However, in these studies, glucose tolerance status may have confounded this relation. Thus, less is known about the role of insulin or proinsulin in the pathway to CVD.

It has been suggested that proinsulin as a molecule might be atherogenic (13). In addition, higher proinsulin levels may also be attributed to β-cell dysfunction (14). Another explanation is that insulin resistance explains the relation of both insulin and proinsulin with (CVD) mortality.

Differences in the predictive value of insulin and proinsulin in the fasting and the postload state are difficult to interpret because of the inherent differences in variability. Insulin and proinsulin concentrations have been shown to be highly variable, particularly in the postload state (15), and random misclassification leads to an underestimation of the true association with CVD. In the present study, in a subsample of the population-based Hoorn Study cohort stratified for glucose tolerance status, fasting and postload insulin and proinsulin levels were determined twice on separate days, 2 weeks apart. Homeostasis model assessment of insulin resistance (HOMA-IR) (16) was used as a marker of insulin resistance. To study the possible role of β-cell dysfunction, a disproportionately elevated proinsulin-to-insulin ratio (17) and homeostasis model assessment of β-cells (HOMA-B) (16) were calculated.

The aim of this study was, first, to investigate the longitudinal association of the mean of duplicate measures of fasting and postload insulin and proinsulin concentrations and insulin resistance, as estimated by HOMA-IR, with all-cause and CVD mortality. The second aim was to study the role of insulin resistance and

From the [1]Institute for Research in Extramural Medicine, VU University Medical Center, Amsterdam, the Netherlands; the [2]Department of General Practice, VU University Medical Center, Amsterdam, the Netherlands; the [3]Department of Internal Medicine, Academic Hospital Maastricht, Maastricht, the Netherlands; and the [4]Department of Endocrinology, VU University Medical Center, Amsterdam, the Netherlands.

Address correspondence and reprint requests to Marjan Alssema, MSc, Institute for Research in Extramural Medicine, VU University Medical Center, Van der Boechorststraat 7, 1081 BT Amsterdam, the Netherlands. E-mail: m.alssema@vumc.nl.

Received for publication 11 October 2004 and accepted in revised form 12 January 2005.

Abbreviations: CVD, cardiovascular disease; HOMA-B, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; PAI-1, plasminogen activator inhibitor-1.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

glucose tolerance status in the relation between insulin or proinsulin and mortality. All analyses were done in a population-based cohort that was stratified for glucose tolerance status.

RESEARCH DESIGN AND METHODS

The Hoorn Study is a population-based cohort study on type 2 diabetes in the general Dutch population. The study population and research design have been described in detail previously (15). In summary, in 1989, 3,553 men and women, aged 50–75 years, were randomly selected from the population register of the middle-sized Dutch town of Hoorn. Of the 2,540 subjects (71.5%) who agreed to participate, 56 non-Caucasians were excluded. Therefore, the study cohort consisted of 2,484 men and women. All subjects gave written informed consent. The Ethics Committee of the VU University Medical Center approved the study. After exclusion of patients with known diabetes ($n = 90$), as defined by use of insulin or blood glucose–lowering agents, or a prescribed diet for diabetes, a subgroup of 1,109 participants from the initial study cohort, stratified by age, sex, and 2-h postload glucose values, was invited for a second oral glucose tolerance test. For reasons of efficiency, insulin and proinsulin were measured in duplicate (~2 weeks apart) in a subsample of 630 participants of the stratified sample of 1,109 participants, including participants with impaired glucose tolerance and all participants with newly detected diabetes (15). Subjects with values below the lower limit of sensitivity of the insulin or proinsulin assay were excluded. For the present analyses, subjects were included if either insulin or proinsulin values were available in duplicate ($n = 605$). Of these 605 subjects, one subject was lost to follow-up. Thus, the present study population comprised 604 subjects. A total of 602 participants had duplicate insulin values, and proinsulin values were available for 511 participants.

Anthropometric measurements were obtained from all participants. Weight was measured while participants were wearing light clothes only. Participants were not wearing shoes while height was measured. BMI was calculated as weight (in kilograms) divided by the square of height (in meters). Waist circumference (centimeters) was measured following a standardized procedure (15).

Systolic and diastolic blood pressure were determined at the right upper-arm, after 5 min of rest in seated participants, with a random-zero sphygmomanometer (Hawksley-Gelman, Lancing, U.K.). The average of duplicate measurements was used.

All laboratory analyses were performed at the VU University Medical Center in Amsterdam. Fasting plasma glucose concentration and 2-h postload plasma glucose concentration were determined by the glucose dehydrogenase method (Merck, Darmstadt, Germany). Immunospecific insulin was measured in serum by a double-antibody radio immunoassay (lot SP21; Linco Research, St. Louis, MO) in which proinsulin and 32,33 split proinsulin cross-reacts by <0.2%. Proinsulin was measured by a double-antibody radioimmunoassay (Lilly Laboratory for Clinical Research, Indianapolis, IN) in which 31,32 proinsulin cross-reacts by 63% (18). Triglycerides, total cholesterol, and HDL cholesterol measured on the first visit were determined by enzymatic techniques (Boehringer-Mannheim, Mannheim, Germany).

Mortality follow-up

There is a continuous follow-up to register mortality from the participants of the Hoorn Study, with the municipal register of the city of Hoorn providing information about the vital status of the participants. Information of causes of death was obtained from medical records of general practitioners and from the local hospital. Causes of death were coded according to the ICD-9 (19). CVD mortality was defined as ICD-9 codes 390–459 (diseases of the circulatory system) or 798 (sudden death, cause unknown) because, generally, sudden death is of cardiovascular origin (20). The mortality follow-up was completed by January 2003.

Statistical methods

Glucose tolerance status (based on fasting and postload glucose values) and fasting and postload insulin and proinsulin values were based on the mean of two measurements. If only one value was determined, values were based on one measurement. This was done for 1 subject for fasting plasma glucose, 16 subjects for postload plasma glucose, 16 subjects for fasting insulin, and 61 subjects for fasting proinsulin.

The mean fasting and postload glucose values of duplicate oral glucose tolerance tests were used to define the glucose tolerance status of the participants (21). Insulin resistance was estimated by HOMA-IR, calculated as fasting insulin (in microunits per milliliter) × fasting glucose (in millimoles per liter)/22.5. β-Cell function was estimated by HOMA-B, calculated as fasting insulin × 20/fasting glucose (in millimoles per liter) − 3.5 (16).

Due to skewed distributions, baseline characteristics are shown as medians with interquartile ranges. Differences in baseline measurements for groups of glucose tolerance status were examined using linear regression for continuous data and logistic regression for dichotomous data, adjusted for age. Correlations among insulin, proinsulin, HOMA-IR, and HOMA-B were calculated by Spearman correlation coefficients because of skewed distribution of insulin and proinsulin.

Hazard ratios (HRs) and 95% CIs were obtained from multivariate Cox proportional hazards models. HRs are calculated for one increase in interquartile range value, which is calculated for each variable. All models were adjusted for age and sex. We tested for possible interaction between insulin, proinsulin, HOMA-IR, and glucose tolerance status by adding two product terms to the survival models insulin × impaired glucose metabolism and insulin × diabetes. We also tested for possible interaction among insulin, proinsulin, HOMA-IR, and sex because this was found in some studies (2). In the second model, we additionally adjusted for glucose tolerance status as a potential confounding factor. Third, we also adjusted for insulin resistance, calculated as HOMA-IR, to investigate the effect of insulin and proinsulin levels on (CVD) mortality independent of HOMA-IR. To find other potential confounding or mediating factors, we added fasting and postload glucose, BMI, triglycerides, HDL cholesterol, total cholesterol, hypertension, and current smoking one by one to the third model. We adjusted our model for the variables that affected the estimated association. A P value <0.05 was considered statistically significant. Testing for interaction, we considered a P value <0.10 statistically significant. Statistical analyses were performed with the SPSS software package for Windows (version 10.1.4).

Table 1—*Baseline characteristics in median values (interquartile range) and mortality in categories of glucose tolerance status* (n = *604*)

	NGM*	IGM	New diabetes
n	277	208	119
Baseline			
Age (years)	63.8 (11.7)	64.7 (12.6)	66.2 (10.4)
Sex (% male)	48.4	48.6	47.9
BMI (kg/m^2)	25.6 (4.2)	27.4 (4.7)	28.1 (5.1)
Waist circumference (cm)	89.5 (13.2)	95.0 (15.0)	99.5 (15.8)
Fasting glucose (mmol/l)†	5.30 (0.55)	6.05 (0.79)	7.40 (1.75)
2-h postload glucose (mmol/l)†	5.75 (2.23)	8.50 (1.54)	12.65 (4.95)
Triglycerides (mmol/l)	1.30 (0.80)	1.70 (1.10)	2.00 (1.50)
HDL cholesterol (mmol/l)	1.35 (0.49)	1.17 (0.47)	1.10 (0.36)
Total cholesterol (mmol/l)	6.60 (1.50)	6.80 (1.50)	6.50 (1.70)
Diastolic blood pressure (mmHg)†	80.0 (13.0)	83.0 (11.5)	84.3 (11.5)
Systolic blood pressure (mmHg)†	130.5 (22.0)	140 (27.4)	144 (20.8)
Hypertension (%)‡	27.5	48.6	55.5
Current smoker (%)	31.4	21.6	21.0
Fasting-specific insulin (pmol/l)†	72.1 (36.8)	88.6 (53.9)	117.2 (70.4)
Fasting proinsulin (pmol/l)†	9.5 (7.9)	14.2 (12.9)	21.7 (20.0)
Proinsulin-to-insulin ratio†	0.12 (0.11)	0.14 (0.13)	0.16 (0.14)
Postload-specific insulin (pmol/l)†	310.5 (260.4)	532.6 (542.7)	600.5 (567.3)
Postload proinsulin (pmol/l)†	49.8 (49.2)	74.9 (63.4)	93.4 (67.2)
HOMA-IR	2.80 (1.55)	4.01 (2.47)	6.61 (4.53)
HOMA-B	137.4 (75.5)	123.2 (85.8)	104.4 (81.3)
11-year follow-up			
All-cause death [*n* (%)]	59 (21.3)	55 (26.4)	42 (35.3)
CVD mortality or sudden death [*n* (%)]	20 (7.2)	22 (10.6)	17 (14.3)

Values within parentheses represent interquartile ranges unless otherwise indicated. Linear regression for continuous variables: $P < 0.05$, except for total cholesterol ($P = 0.98$). Logistic regression for percentages: $P < 0.05$, except for sex ($P = 0.69$) and CVD mortality ($P = 0.10$). *Glucose tolerance status based on the mean fasting and 2-h postload glucose levels of two oral glucose tolerance tests; †based on the mean of two measurements; ‡hypertension (diastolic blood pressure ≥95 mmHg or systolic blood pressure ≥160 mmHg or medication). NGM, normal glucose metabolism (fasting glucose <6.1 mmol/l and 2-h glucose <7.8 mmol/l); IGM, impaired glucose metabolism (fasting glucose ≥6.1 and <7.0 mmol/l or 2-h glucose ≥7.8 and <11.1 mmol/l); diabetes, fasting glucose ≥7.0 mmol/l or 2-h glucose ≥11.1 mmol/l; HOMA-IR, fasting insulin (in microunits per milliliter) × fasting glucose (in millimoles per liter)/22.5; HOMA-B, fasting insulin (in microunits per milliliter) × 20/fasting glucose (in millimoles per liter) − 3.5.

RESULTS — The characteristics of the study cohort, stratified for glucose tolerance status, are shown in Table 1. Of the 604 participants, 156 died, of whom 59 died from CVD or sudden death during the 11 years of follow-up. There was an increasing risk of all-cause mortality over the glucose tolerance groups ($P = 0.04$). For CVD mortality, increase in risk over glucose tolerance groups was not statistically significant ($P = 0.10$). All other variables, except sex and total cholesterol, showed statistically significant differences ($P < 0.05$) across glucose tolerance groups.

Spearman correlation coefficients among insulin, proinsulin, HOMA-IR, and HOMA-B are shown in Table 2. All shown variables were statistically significant and correlated with each other ($P < 0.01$), except fasting proinsulin with HOMA-B and proinsulin-to-insulin ratio with postload insulin and with HOMA-IR. Proinsulin-to-insulin ratio was statistically significant but inversely related to fasting insulin and HOMA-B.

HRs for fasting insulin, fasting proinsulin, and HOMA-IR, adjusted for age and

Table 2—*Spearman correlation coefficients for insulin and proinsulin, HOMA-IR, and HOMA-B*

	Fasting-specific insulin	Fasting proinsulin	Proinsulin-to-insulin ratio	Postload-specific insulin	Postload proinsulin	HOMA-IR
Fasting proinsulin	0.47*					
Proinsulin-to-insulin ratio	0.14*	0.78*				
Postload-specific insulin	0.67*	0.43*	0.04			
Postload proinsulin	0.51*	0.68*	0.41*	0.74*		
HOMA-IR	0.95*	0.53*	−0.04	0.63*	0.52*	
HOMA-B	0.63*	0.09	−0.34*	0.44*	0.25*	0.38*

*Correlation is statistically significant ($P < 0.01$). HOMA-IR, fasting insulin (in microunits per milliliter) × fasting glucose (in millimoles per liter)/22.5; HOMA-B, fasting insulin (in microunits per milliliter) × 20/fasting glucose (in millimoles per liter) − 3.5.

Table 3—*Hazard ratios for fasting and postload insulin and proinsulin, HOMA-IR, and HOMA-B* (n = 604)

	IR value	HR per increase in IR (95% CI) for all-cause mortality	HR per increase in IR (95% CI) for CVD mortality
Fasting-specific insulin	51.6 pmol/l		
Model 1		1.15 (0.98–1.35)	1.14 (0.87–1.49)
Model 2		1.09 (0.91–1.31)	1.04 (0.77–1.40)
Model 3		1.00 (0.71–1.41)	0.92 (0.53–1.60)
Model 1 + proinsulin		1.00 (0.81–1.22)	0.90 (0.65–1.25)
Fasting proinsulin	12.8 pmol/l		
Model 1		1.21 (1.04–1.42)	1.33 (1.06–1.66)
Model 2		1.19 (1.00–1.40)	1.27 (1.00–1.63)
Model 3		1.21 (1.00–1.46)	1.34 (1.01–1.76)
Model 3 + triglycerides		1.18 (0.97–1.43)	1.29 (0.97–1.72)
Model 3 + hypertension*		1.19 (0.98–1.44)	1.31 (0.99–1.73)
Model 3 + current smoking		1.23 (1.01–1.49)	1.37 (1.03–1.81)
Proinsulin-to-insulin ratio	0.12		
Model 1		1.11 (0.96–1.28)	1.19 (0.97–1.47)
Model 2		1.10 (0.95–1.28)	1.17 (0.95–1.45)
Model 3		1.11 (0.95–1.29)	1.18 (0.95–1.47)
Postload-specific insulin	408 pmol/l		
Model 1		1.06 (0.95–1.19)	1.10 (0.93–1.31)
Model 2		1.03 (0.91–1.17)	1.06 (0.87–1.28)
Model 3		1.01 (0.88–1.16)	1.04 (0.85–1.28)
Postload proinsulin	57.5 pmol/l		
Model 1		1.12 (0.92–1.37)	1.26 (0.93–1.70)
Model 2		1.07 (0.87–1.33)	1.16 (0.84–1.61)
Model 3		1.06 (0.85–1.31)	1.15 (0.82–1.60)
HOMA-IR	2.82		
Model 1		1.16 (1.01–1.32)	1.16 (0.94–1.44)
Model 2		1.10 (0.93–1.30)	1.07 (0.82–1.40)
Model 3		—	—
Model 1 + proinsulin		1.00 (0.83–1.20)	0.97 (0.73–1.29)
HOMA-B	82.3		
Model 1		0.99 (0.83–1.19)	0.94 (0.69–1.28)
Model 2		1.03 (0.86–1.24)	0.99 (0.73–1.35)
Model 3		0.99 (0.81–1.21)	0.95 (0.68–1.34)

HOMA-IR, fasting insulin (in microunits per milliliter) × fasting glucose (in millimoles per liter)/22.5; HOMA-B, fasting insulin (in microunits per milliliter) × 20/fasting glucose (in millimoles per liter) − 3.5. *Hypertension: Diastolic blood pressure ≥95 mmHg or systolic blood pressure ≥160 mmHg or medication. Model 1: Adjusted for age and sex. Model 2: Adjusted for age, sex, and glucose tolerance status. Model 3: Adjusted for age, sex, glucose tolerance status, and HOMA-IR. IR, interquartile range.

sex, were calculated for separate strata of glucose tolerance. The HRs for insulin and HOMA-IR were the highest in the normal glucose metabolism group (data not shown). Proinsulin was associated with mortality in all groups of glucose tolerance status. Since the tests for interactions among insulin, proinsulin, HOMA-IR, and glucose tolerance status were not statistically significant ($P > 0.10$), these groups were combined for subsequent analyses.

In Table 3, HRs per increase in interquartile range, calculated from the present study population for both all-cause and CVD mortality, are shown. Fasting insulin, proinsulin-to-insulin ratio, postload insulin and proinsulin levels, and HOMA-B were not significantly associated with all-cause or CVD mortality. Fasting proinsulin, however, was significantly associated with all-cause mortality (HR 1.21 [95% CI 1.04–1.42]) and CVD mortality (1.33 [1.06–1.66]), adjusting for age and sex. After adjustment for glucose tolerance status and additionally for HOMA-IR, the HRs for proinsulin were only slightly attenuated and remained significant. HOMA-IR was associated with all-cause mortality (1.16 [1.01–1.32]) but not with CVD mortality. By including fasting proinsulin in the model of HOMA-IR, the association between HOMA-IR and all-cause mortality disappeared (1.00 [0.83–1.20]). Also, by including proinsulin in the model of insulin, HRs for insulin became smaller (Table 3), whereas HRs for proinsulin did not substantially change (data not shown).

In addition, we tested for other potential confounding risk factors (Table 3) in the relation between proinsulin and (CVD) mortality. Only triglycerides and hypertension slightly attenuated the association between proinsulin and all-cause mortality. Other risk factors, i.e., fasting and postload glucose, BMI, HDL cholesterol, total cholesterol, and HOMA-B, did not change the association between proinsulin and mortality (data not shown). The associations with mortality did not differ between men and women (data not shown).

CONCLUSIONS — In the present study, in a subsample of a population-based prospective study, we found that proinsulin was an independent risk factor for both all-cause and CVD mortality, also after adjusting for both insulin resistance and glucose tolerance status.

Fasting proinsulin per increase in interquartile range was associated with a 21% higher all-cause mortality and a 33% higher CVD mortality. The association of proinsulin with mortality risk appeared stronger than that of insulin with mortality risk. This has also been observed in another longitudinal study (9) and in cross-sectional studies (22,23). Moreover, in a model that included both proinsulin and insulin or HOMA-IR, proinsulin remained significantly associated with (CVD) mortality, whereas insulin or HOMA-IR did not remain statistically significant. Only one previous study considered insulin as a covariate (11). In that study, proinsulin remained an independent risk factor for stroke, and the excess risk of insulin disappeared when proinsulin was added to the model.

High proinsulin levels can be a result of insulin resistance or β-cell failure, and both may contribute to the association between proinsulin and CVD. Proinsulin has been shown to be strongly associated

with insulin resistance in a recent study (24), which may at least partly explain the association with CVD and mortality (25,26). A strong correlation between proinsulin and HOMA-IR can cause multicollinearity when both variables are included in the same model. In the present study, the Spearman correlation coefficient between proinsulin and HOMA-IR was 0.53, substantially lower than the 0.90 cutoff value, which is often used as a rule of thumb to define multicollinearity. We found that the association between proinsulin and (CVD) mortality was independent of insulin resistance. In contrast, the association between HOMA-IR and CVD mortality disappeared following adjustment for proinsulin. Therefore, high proinsulin levels might be an intermediate factor in the association between insulin resistance and CVD risk, in which high proinsulin levels are more closely related to CVD than insulin resistance.

High proinsulin levels may also be attributed to β-cell dysfunction or an increased demand on the β-cells (14). In the current study, disproportionately elevated proinsulin-to-insulin ratios or HOMA-B (16) were not associated with an increased risk of CVD mortality, suggesting that proinsulin per se may contribute to the atherogenic or thrombogenic process. Indeed, a clinical study in which human proinsulin was administered for at least 1 year was stopped when six patients treated with human proinsulin had adverse cardiovascular events (13), whereas subjects treated with insulin had no adverse events.

In the IRAS (Insulin Resistance Atherosclerosis Study), plasminogen activator inhibitor-1 (PAI-1) was suggested to play a role in the association between proinsulin and CVD (22). Increased PAI-1 activity promotes both fibrosis and thrombosis and is associated with CVD (27). It was already shown that PAI-1 activity increased after insulin or proinsulin was administered in vitro (28) and in vivo (29). Unfortunately, in the Hoorn Study, data on PAI-1 are not available. PAI-1 levels are elevated in subjects with insulin resistance, impaired glucose metabolism, and the metabolic syndrome (27). Therefore, the metabolic syndrome, which is defined by high triglycerides, impaired glucose metabolism, hypertension, abdominal obesity, and/or low HDL (30), is another potential confounding factor in the relation between proinsulin and CVD risk. However, in our data, triglyceride concentration, glucose tolerance status, and hypertension explained only a small part of the association between proinsulin and CVD risk.

Hyperinsulinemia has been shown to predict all-cause (1) and CVD mortality (2,4,5,8) in the general population. We did find an association between insulin and CVD mortality, similar to the findings in the meta-analysis of the DECODE (Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe) Insulin Study Group. However, in the present study, the association between insulin and CVD mortality was not significant, probably because of the relatively small number of case subjects.

In a number of studies, the association between insulin and coronary heart disease was J-shaped (3) or U-shaped (31). In the present subpopulation of the Hoorn Study, the number of cases was too small to explore this association. When we analyzed the entire Hoorn Study population, baseline insulin levels were available for 1,806 participants with normal glucose metabolism. Indeed, the HRs for fasting-specific insulin of the lowest quintile against the middle three quintiles, adjusted for age and sex, were 1.10 (95% CI 0.81–1.50) for all-cause mortality and 1.07 (0.65–1.75) for CVD mortality (unpublished data). For the present analysis, we did not consider this a relevant deviation of a linear association.

In some studies, postload insulin levels, 2 h after a 75-g glucose load, have been associated with coronary artery disease (7,8). In the present study, postload insulin levels were not significantly associated with mortality, and the association was weaker than between fasting insulin and mortality. These results are consistent with findings from a recent meta-analysis (2). The weaker association between postload insulin and mortality was explained by the larger biological variation of postload insulin levels. In our study, biological variation was reduced by measuring insulin levels in duplicate, but this could still play a role in attenuating the association between postload insulin and mortality.

We did not use gold standard techniques for measuring insulin sensitivity (i.e., the hyperinsulinemic-euglycemic clamp) and for β-cell function (i.e., the hyperglycemic clamp) (32). Instead, HOMA-IR was used as an estimate of insulin resistance (16). In previous studies, HOMA-IR was found to be a predictor of CVD in diabetic patients (25,33). However, HOMA-IR cannot be used to estimate insulin resistance in diabetic patients (34). Therefore, patients with known diabetes were excluded from the present study. Our observation that insulin levels and HOMA-IR were the strongest predictors of all-cause and CVD mortality in subjects with a normal glucose tolerance status might be explained by the fact that insulin and HOMA-IR are less accurate indexes of insulin resistance in patients with impaired glucose metabolism or newly detected type 2 diabetes.

A strength of our study is that levels of insulin, proinsulin, and glucose were measured twice to improve the precision. We included participants with newly diagnosed diabetes and with impaired glucose metabolism to enlarge the range of distribution of insulin, proinsulin, HOMA-IR, and HOMA-B. Also, these subjects are especially important to include because of their enhanced CVD risk.

In our study, proinsulin concentration was associated with both all-cause and CVD mortality, independent of glucose tolerance status and insulin resistance. In addition, we showed that the association between HOMA-IR and all-cause mortality disappeared when proinsulin was entered into the model. High proinsulin levels might therefore play a role in the atherothrombotic process, possibly as an intermediate in the association between insulin resistance and CVD risk.

Acknowledgments— M.A. was supported by the Dutch Diabetes Research Foundation (DFN grant no. 2001.00.052).

We gratefully acknowledge Corry Popp-Snijders for performing analyses of insulin-like molecules and Manon van Hecke for her work on the follow-up of the cohort.

References

1. Pyorala M, Miettinen H, Laakso M, Pyorala K: Plasma insulin and all-cause, cardiovascular, and noncardiovascular mortality: the 22-year follow-up results of the Helsinki Policemen Study. *Diabetes Care* 23:1097–1102, 2000
2. Hu G, Qiao Q, Tuomilehto J, Eliasson M, Feskens EJ, Pyorala K: Plasma insulin and cardiovascular mortality in non-diabetic European men and women: a meta-analysis of data from eleven prospective stud-

ies. *Diabetologia* 47:1245–1256, 2004
3. Folsom AR, Szklo M, Stevens J, Liao F, Smith R, Eckfeldt JH: A prospective study of coronary heart disease in relation to fasting insulin, glucose, and diabetes: the Atherosclerosis Risk in Communities (ARIC) study. *Diabetes Care* 20:935–942, 1997
4. Lakka HM, Lakka TA, Tuomilehto J, Sivenius J, Salonen JT: Hyperinsulinemia and the risk of cardiovascular death and acute coronary and cerebrovascular events in men: the Kuopio Ischaemic Heart Disease Risk Factor Study. *Arch Intern Med* 160:1160–1168, 2000
5. Lehto S, Ronnemaa T, Pyorala K, Laakso M: Cardiovascular risk factors clustering with endogenous hyperinsulinaemia predict death from coronary heart disease in patients with type II diabetes. *Diabetologia* 43:148–155, 2000
6. Despres JP, Lamarche B, Mauriege P, Cantin B, Dagenais GR, Moorjani S, Lupien PJ: Hyperinsulinemia as an independent risk factor for ischemic heart disease. *N Engl J Med* 334:952–957, 1996
7. Baltali M, Korkmaz ME, Kiziltan HT, Muderris IH, Ozin B, Anarat R: Association between postprandial hyperinsulinemia and coronary artery disease among non-diabetic women: a case control study. *Int J Cardiol* 88:215–221, 2003
8. Ruige JB, Assendelft WJ, Dekker JM, Kostense PJ, Heine RJ, Bouter LM: Insulin and risk of cardiovascular disease: a meta-analysis. *Circulation* 97:996–1001, 1998
9. Zethelius B, Byberg L, Hales CN, Lithell H, Berne C: Proinsulin is an independent predictor of coronary heart disease: report from a 27-year follow-up study. *Circulation* 105:2153–2158, 2002
10. Yudkin JS, May M, Elwood P, Yarnell JW, Greenwood R, Davey SG: Concentrations of proinsulin like molecules predict coronary heart disease risk independently of insulin: prospective data from the Caerphilly Study. *Diabetologia* 45:327–336, 2002
11. Lindahl B, Dinesen B, Eliasson M, Roder M, Hallmans G, Stegmayr B: High proinsulin levels precede first-ever stroke in a nondiabetic population. *Stroke* 31:2936–2941, 2000
12. Oh JY, Barrett-Connor E, Wedick NM: Sex differences in the association between proinsulin and intact insulin with coronary heart disease in nondiabetic older adults: the Rancho Bernardo Study. *Circulation* 105:1311–1316, 2002
13. Galloway JA, Hooper SA, Spradlin CT, Howey DC, Frank BH, Bowsher RR, Anderson JH: Biosynthetic human proinsulin: review of chemistry, in vitro and in vivo receptor binding, animal and human pharmacology studies, and clinical trial experience. *Diabetes Care* 15:666–692, 1992
14. Yudkin JS: Circulating proinsulin-like molecules. *J Diabetes Complications* 7: 113–123, 1993
15. Mooy JM, Grootenhuis PA, de Vries H, Kostense PJ, Popp-Snijders C, Bouter LM, Heine RJ: Intra-individual variation of glucose, specific insulin and proinsulin concentrations measured by two oral glucose tolerance tests in a general Caucasian population: the Hoorn Study. *Diabetologia* 39:298–305, 1996
16. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419, 1985
17. Haffner SM, Mykkanen L, Valdez RA, Stern MP, Holloway DL, Monterrosa A, Bowsher RR: Disproportionately increased proinsulin levels are associated with the insulin resistance syndrome. *J Clin Endocrinol Metab* 79:1806–1810, 1994
18. Nijpels G, Popp-Snijders C, Kostense PJ, Bouter LM, Heine RJ: Fasting proinsulin and 2-h postload glucose levels predict the conversion to NIDDM in subjects with impaired glucose tolerance: the Hoorn Study. *Diabetologia* 39:113–118, 1996
19. Kupka K: *International Classification of Diseases: Ninth Revision. WHO Chron* 32: 219–225, 1978
20. Kannel WB, Plehn JF, Cupples LA: Cardiac failure and sudden death in the Framingham Study. *Am Heart J* 115: 869–875, 1988
21. Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 15:539–553, 1998
22. Haffner SM, D'Agostino R, Mykkanen L, Hales CN, Savage PJ, Bergman RN, O'Leary D, Rewers M, Selby J, Tracy R, Saad MF: Proinsulin and insulin concentrations in relation to carotid wall thickness: Insulin Resistance Atherosclerosis Study. *Stroke* 29:1498–1503, 1998
23. Bokemark L, Wikstrand J, Wedel H, Fagerberg B: Insulin, insulin propeptides and intima-media thickness in the carotid artery in 58-year-old clinically healthy men: the Atherosclerosis and Insulin Resistance study (AIR). *Diabet Med* 19:144–151, 2002
24. Pfutzner A, Kunt T, Hohberg C, Mondok A, Pahler S, Konrad T, Lubben G, Forst T: Fasting intact proinsulin is a highly specific predictor of insulin resistance in type 2 diabetes. *Diabetes Care* 27:682–687, 2004
25. Bonora E, Formentini G, Calcaterra F, Lombardi S, Marini F, Zenari L, Saggiani F, Poli M, Perbellini S, Raffaelli A, Cacciatori V, Santi L, Targher G, Bonadonna R, Muggeo M: HOMA-estimated insulin resistance is an independent predictor of cardiovascular disease in type 2 diabetic subjects: prospective data from the Verona Diabetes Complications Study. *Diabetes Care* 25:1135–1141, 2002
26. Hedblad B, Nilsson P, Engstrom G, Berglund G, Janzon L: Insulin resistance in non-diabetic subjects is associated with increased incidence of myocardial infarction and death. *Diabet Med* 19:470–475, 2002
27. Lyon CJ, Hsueh WA: Effect of plasminogen activator inhibitor-1 in diabetes mellitus and cardiovascular disease (Review). *Am J Med* 115 (Suppl. 8A):62S–68S, 2003
28. Nordt TK, Schneider DJ, Sobel BE: Augmentation of the synthesis of plasminogen activator inhibitor type-1 by precursors of insulin: a potential risk factor for vascular disease. *Circulation* 89:321–330, 1994
29. Nordt TK, Sawa H, Fujii S, Sobel BE: Induction of plasminogen activator inhibitor type-1 (PAI-1) by proinsulin and insulin in vivo. *Circulation* 91:764–770, 1995
30. Grundy SM, Brewer HB, Jr, Cleeman JI, Smith SC, Jr, Lenfant C: Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Arterioscler Thromb Vasc Biol* 24:e13–e18, 2004
31. Balkau B, Eschwege E: Insulin resistance: an independent risk factor for cardiovascular disease? (Review). *Diabetes Obes Metab* (Suppl.1):S23–S31, 1999
32. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979
33. Yip J, Facchini FS, Reaven GM: Resistance to insulin-mediated glucose disposal as a predictor of cardiovascular disease. *J Clin Endocrinol Metab* 83:2773–2776, 1998
34. Laakso M: How good a marker is insulin level for insulin resistance? *Am J Epidemiol* 137:959–965, 1993