Proinsulin Is an Independent Predictor of Coronary Heart Disease
Report From a 27-Year Follow-Up Study

Björn Zethelius, MD; Liisa Byberg, MSc; C. Nicholas Hales, PhD; Hans Lithell, MD; Christian Berne, MD

Background—Some, but not all, studies have reported a relationship between plasma insulin and coronary heart disease (CHD). Conventional nonspecific insulin assays are also measuring various fractions of proinsulin-like molecules due to cross-reactivity. The long-term relationship between proinsulin-like molecules and CHD is largely unknown. For this reason, the longitudinal relationships between intact proinsulin, split proinsulin, specific insulin, immunoreactive insulin, and CHD, were studied in a population-based cohort of 50-year-old men (n = 874), with a follow-up of 27 years.

Methods and Results—Fasting proinsulin-like molecule and specific-insulin concentrations were measured in plasma (stored frozen since baseline 1970 to 1973) by specific 2-site immunometric assays. Immunoreactive insulin concentrations were determined at baseline. The associations between proinsulin-like molecules, specific insulin, immunoreactive insulin, and CHD mortality (International Classification of Diseases [9th revision] codes 410 to 414) were analyzed using Cox’s proportional hazards regression and presented as hazard ratios (HRs) with their 95% confidence intervals (CIs) for a 1-SD increase in a predictor variable. In the univariate analysis, intact proinsulin (HR, 1.69; 95% CI, 1.41 to 2.01) was the strongest predictor of death from CHD. In the multivariate analysis, smoking (HR, 1.57; 95% CI, 1.03 to 2.38), intact proinsulin (HR, 1.47; 95% CI, 1.18 to 1.82), systolic blood pressure (HR, 1.38; 95% CI, 1.14 to 1.66), and LDL/HDL cholesterol ratio (HR, 1.31; 95% CI, 1.12 to 1.53) were independent predictors of CHD mortality (adjusted for body mass index, triglycerides, and fasting glucose), whereas specific insulin and immunoreactive insulin were not (HR, 1.12; 95% CI, 0.90 to 1.40). The increased risk was restricted to the upper third of the proinsulin distribution.

Conclusion—Increased proinsulin concentrations predict death and morbidity caused by CHD over a period of 27 years, independent of other major cardiovascular risk factors. (Circulation. 2002;105:2153-2158.)

Key Words: insulin • risk factors • coronary heart disease • myocardial infarction • epidemiology

Obesity and type 2 diabetes are conditions associated with both insulin resistance and an increased risk of coronary heart disease (CHD). It is not known to what extent insulin resistance per se, the compensatory increase in insulin, or both contribute to the risk of CHD. Increased plasma immunoreactive insulin (IRI) concentrations constitute an independent risk factor for CHD over follow-up periods of ~10 years; however, the relationship became attenuated or nonsignificant after longer time periods, up to 22 years.

Insulin radioimmunoassays usually fail to discriminate between insulin and proinsulin-like molecules (PLM; ie, intact proinsulin and its split products). Therefore, it has been suggested that increased concentrations of insulin precursor molecules, rather than plasma insulin per se, constitute the association with CHD.

The extent to which PLMs contribute to the association between IRI and CHD is largely unknown. In a longitudinal study lasting up to 6.5 years, the significant relationship between proinsulin and CHD became nonsignificant when controlled for the confounding effect of body weight.

The primary aim of this prospective population-based study was to determine the longitudinal relationship between the plasma concentrations of intact proinsulin, 32-33 split proinsulin, specific insulin, IRI, and death from cardiovascular causes, and to determine if the predictor by outcome associations affected all-cause mortality. A secondary aim was to perform a similar analysis for morbidity owing to specified cardiovascular outcomes.

Methods

Subjects
In 1970, all men born from 1920 to 1924 and living in Uppsala, Sweden (n = 2841), were invited to a health survey, in which 82%
(n=2322) participated. Concentrations of proinsulin-like molecules (PLM) were determined in baseline plasma samples from a subset of 1306 subjects (all of which were available after a random loss of ~1000 samples because of a freezer failure). There was no selection bias for subjects whose proinsulin concentrations were determined. The present study was based on subjects with complete data from the baseline investigation (n=1047). To select men free from cardiovascular disease (CVD) and cancer at baseline (n=874), 173 men were excluded because of the presence of previous angina pectoris or myocardial infarction (MI), Q or QS-complexes or left bundle branch block (Minnesota codes 1.1 to 1.3 or 7.1, respectively) in the baseline ECG registration, current treatment with nitroglycerine or digalis, previous or incident CVD within 1 year after baseline (International Classification of Diseases, 9th revision [ICD-9], codes 390 to 459), or previous or incident malignant cancer within 2 years after baseline (ICD-9 codes 140 to 208 or 230 to 239).

Information concerning previous disease and current pharmacological treatment was collected by a medical questionnaire and through physician interview. Information concerning mortality and morbidity from incident disease was collected from official Swedish registries held by the Center for Epidemiology, National Board of Health and Welfare in Sweden.

Outcome Definitions
Outcome and survival-time variables were defined using the registry data (censor date, December 31, 1996). Mortality was defined as death, recorded in the Cause of Death Registry (CDR) and morbidity as first time hospitalized, as recorded in the In-Patient Registry or recording in the CDR or first registration in the Swedish Cancer Registry, owing to the following causes: (1) MI (ICD-9 code 410), (2) CHD (ICD-9 codes 410 to 414), (3) CVD (ICD-9 codes 390 to 459), and (4) all causes (any ICD-9 code). A quality control of the CDR by the Swedish centers of the World Health Organization MONItoring trends and determinants in CArdiovascular disease (MONICA) study showed good agreement for registration of MI. None of the subjects had missing registry data.

Baseline Characteristics
The concentrations of intact proinsulin and 32-33 split proinsulin were determined between 1995 and 1998 by the 2-site immunometric assay technique in plasma samples (n=1306) that had been stored frozen (~70°C) since baseline. Specific insulin concentrations were also determined in these samples by the Access Immunnoassay System (Sanofi Pasteur Diagnostics). Analyses were performed, blinded for outcome, at the Department of Clinical Biochemistry, Addenbrooke’s Hospital, Cambridge, United Kingdom. At baseline, the serum IRI concentrations were determined with the Phadebas Insulin Test (Pharmacia AB). A comparison between IRI and the sum of specific insulin and the PLMs by use of a Bland-Altman plot (Figure 1) did not disclose any range-specific deviations, ie, no bias-trend (trend for the mean difference to rise or fall with increasing concentrations; r=0.019, P=0.66). The absolute values of the sum of the 3 specific measurements were not affected in a nonlinear way in relation to baseline IRI determinations.

Serum total, LDL and HDL cholesterol and triglyceride concentrations, fasting blood glucose concentrations, systolic (SBP) and diastolic blood pressure, weight, and height were measured under standardized conditions according to the baseline protocol. Body mass index was calculated as weight/height squared (kg/m²). Smoking status was established during the baseline interview with a physician.

The Ethics Committee of the Faculty of Medicine at Uppsala University approved the study. Informed consent was obtained from all participants.

Statistical Analyses
Skewed variables were log transformed to reach normal distribution. Normally distributed variables were used in all statistical analyses. The statistical software package STATA 5.0 for the personal computer (STATA Corporation) was used. All tests were 2-tailed, and P<0.05 was considered statistically significant. Cox’s proportional hazards regression analyses were used to determine the magnitude and the statistical significance of the relationships between the predictors, as standardized variables, and each of the defined outcome variables. All analyses were adjusted for age at baseline. In the multivariate models, adjustments were made for known major risk factors, chosen based on the strength of the bivariate associations between these factors and CHD death (Table 1), office SBP, LDL/HDL cholesterol ratio, and smoking status, and for the possible confounding effects of body mass index, fasting concentrations of blood glucose, and serum triglycerides. Results are presented as hazard ratios (HRs) and their 95% confidence intervals (CIs).

Results
Baseline clinical characteristics of the entire study population (n=874) and standardized HRs for fatal CHD (n=107) over the 26.7-year follow-up period are shown in Table 1.

| TABLE 1. Clinical Characteristics at Baseline for the Entire Study Population and Hazard Ratios for Fatal Coronary Heart Disease (n=107) Over the 26.7-Year Follow-Up Period |
|---------------------------------|-----------------|-----------------|
| Mean±SD (n=874) Hazard Ratio (95% CI) |
| Intact proinsulin, pmol/L | 3.0±3.2 | 1.69 (1.41–2.01) |
| 32-33 Split proinsulin, pmol/L | 7.1±7.0 | 1.44 (1.21–1.72) |
| Specific insulin, pmol/L | 48.2±34.9 | 1.32 (1.11–1.58) |
| Immunoreactive insulin, pmol/L | 76.5±42.1 | 1.45 (1.21–1.73) |
| Systolic blood pressure, mm Hg | 132±17 | 1.60 (1.37–1.86) |
| Diastolic blood pressure, mm Hg | 83±10 | 1.55 (1.31–1.83) |
| Total cholesterol, mmol/L | 7.0±1.3 | 1.38 (1.19–1.60) |
| LDL-cholesterol, mmol/L | 5.3±1.3 | 1.38 (1.19–1.60) |
| HDL-cholesterol, mmol/L | 1.4±0.4 | 0.67 (0.53–0.84) |
| LDL/HDL cholesterol ratio | 4.3±1.9 | 1.40 (1.26–1.54) |
| Serum triglycerides, mmol/L | 1.9±1.1 | 1.47 (1.24–1.74) |
| Blood glucose, mmol/L | 5.0±0.7 | 1.22 (1.04–1.44) |
| Body mass index, kg/m² | 24.9±3.1 | 1.47 (1.25–1.73) |
| Smoking, % | 53 | 1.82 (1.22–2.71) |

Values are arithmetic means±SD. Hazard ratios from Cox’s proportional hazards regression were applied to variables standardized to 1 SD (except smoking) and adjusted for age at entry.
Mortality

Of the insulin/PLMs, intact proinsulin showed the strongest relationship to death from CHD, and specific insulin showed the weakest, whereas IRI showed a relationship of intermediate magnitude (Table 1). The association between intact proinsulin and death from CHD was only slightly reduced when adjustments were made for the confounding factors listed above (Table 2). The same pattern emerged for death from MI, CHD, and CVD when adjustments were made for the confounding factors described in Statistical Analyses. In this multivariate model, significant predictors of CHD mortality other than intact proinsulin (HR, 1.47; 95% CI, 1.18 to 1.82) were smoking (HR, 1.57; 95% CI, 1.03 to 2.38), SBP (HR, 1.38; 95% CI, 1.14 to 1.66), and LDL/HDL cholesterol ratio (HR, 1.31; 95% CI, 1.12 to 1.53). In contrast to intact proinsulin, the relationship between 32-33 split proinsulin, specific insulin, IRI, and death from CHD became nonsignificant when adjustments were made for the confounding factors listed above (Table 2). The same pattern emerged for death from MI and CVD (Table 2). Smoking, SBP, and the LDL/HDL cholesterol ratio were significant predictors of death from MI, CHD, and CVD in all analyses.

After adjustment for confounders, intact proinsulin also had a significant association with all-cause mortality (Table 2). The relationship between intact proinsulin and death from MI was the strongest, whereas in descending order, relationships were weaker between intact proinsulin and death from CHD, CVD, or all-cause mortality (Table 2). This suggests that the association between intact proinsulin and all-cause mortality is mainly dependent on the relationship between intact proinsulin and death from MI and CHD.

In a separate analysis, on a subset with prevalent and incident diabetes during the follow-up excluded, the relationships between proinsulin and death from the defined outcomes remained significant (Table 2).

Unadjusted Kaplan-Meier survival curves (Figure 2) are presented for death from CHD during the 27 years of monitoring by tertiles of proinsulin concentrations. The risk of death was significantly higher in the highest tertile (proinsulin >3.1 pmol/L), compared with the lowest tertile (proinsulin <1.7 pmol/L). The survival curves diverged after 5 years.

**Figure 2.** Kaplan-Meier survival curves for death from coronary heart disease during 27 years of monitoring, by tertiles of proinsulin concentration at baseline. The risk of death was significantly higher for men in the highest tertile (T3), with proinsulin concentration >3.1 pmol/L, compared with those in the lowest tertile (T1), with proinsulin concentration <1.7 pmol/L. P<0.001.
TABLE 3. Hazard Ratios* for Morbidity According to Concentrations of Proinsulin-Like Molecules and Specific and Immunoreactive Insulin at Baseline

<table>
<thead>
<tr>
<th></th>
<th>MI</th>
<th>CHD</th>
<th>CVD</th>
<th>All Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main data set (n=874)</td>
<td>(n=159)</td>
<td>(n=219)</td>
<td>(n=405)</td>
<td>(n=780)</td>
</tr>
<tr>
<td>Intact proinsulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>1.75 (1.51–2.04)</td>
<td>1.62 (1.43–1.86)</td>
<td>1.33 (1.21–1.46)</td>
<td>1.14 (1.06–1.23)</td>
</tr>
<tr>
<td>Adjusted†</td>
<td>1.66 (1.39–1.98)</td>
<td>1.45 (1.25–1.69)</td>
<td>1.25 (1.12–1.39)</td>
<td>1.12 (1.03–1.21)</td>
</tr>
<tr>
<td>32-33 Split proinsulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>1.43 (1.23–1.66)</td>
<td>1.42 (1.25–1.61)</td>
<td>1.21 (1.12–1.39)</td>
<td>1.09 (1.01–1.18)</td>
</tr>
<tr>
<td>Adjusted†</td>
<td>1.24 (1.03–1.48)</td>
<td>1.18 (1.00–1.38)</td>
<td>1.07 (0.96–1.20)</td>
<td>1.05 (0.96–1.14)</td>
</tr>
<tr>
<td>Specific insulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>1.20 (1.03–1.39)</td>
<td>1.21 (1.07–1.38)</td>
<td>1.08 (0.98–1.19)</td>
<td>1.06 (0.98–1.13)</td>
</tr>
<tr>
<td>Adjusted†</td>
<td>0.94 (0.78–1.14)</td>
<td>0.90 (0.77–1.07)</td>
<td>0.90 (0.80–1.01)</td>
<td>0.99 (0.91–1.08)</td>
</tr>
<tr>
<td>Immunoreactive insulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>1.29 (1.11–1.51)</td>
<td>1.25 (1.09–1.42)</td>
<td>1.12 (1.01–1.23)</td>
<td>1.07 (0.99–1.15)</td>
</tr>
<tr>
<td>Adjusted†</td>
<td>1.07 (0.88–1.29)</td>
<td>0.98 (0.84–1.14)</td>
<td>0.97 (0.86–1.08)</td>
<td>1.00 (0.93–1.10)</td>
</tr>
<tr>
<td>Subset: subjects with diabetes mellitus excluded (n=791)</td>
<td>(n=142)</td>
<td>(n=191)</td>
<td>(n=364)</td>
<td>(n=713)</td>
</tr>
<tr>
<td>Intact proinsulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted†</td>
<td>1.68 (1.39–2.02)</td>
<td>1.52 (1.29–1.79)</td>
<td>1.26 (1.12–1.41)</td>
<td>1.11 (1.02–1.21)</td>
</tr>
</tbody>
</table>

*Hazard ratios with 95% CIs from Cox’s proportional hazards regression were applied to variables standardized to 1 SD (except smoking) and adjusted for age at entry.
†Adjusted for age at entry, body mass index, systolic office blood pressure, smoking status, fasting concentrations of glucose, serum triglycerides, and LDL/HDL cholesterol ratio. MI indicates myocardial infarction (ICD-9 code 410); CHD, coronary heart disease (410 to 414); and CVD, cardiovascular disease (390 to 459).

Morbidity

Intact proinsulin showed the strongest association also with CHD morbidity, and specific insulin showed the weakest, with IRI showing an association with CHD of intermediate magnitude (Table 3). Focusing on the insulin/proinsulin molecules, the strength of the relationship between intact proinsulin and CHD morbidity was only slightly reduced when adjustments were made for a selection of confounding factors as described in Statistical Analyses.

The association between 32-33 split proinsulin and CHD morbidity also remained significant after adjustment for confounders, whereas the associations between specific insulin and CHD morbidity and between and IRI and CHD morbidity did not.

In a separate analysis, on a subset with prevalent and incident diabetes during the follow-up excluded,9,13,14 the relationships between proinsulin and morbidity from the defined outcomes remained significant (Table 3).

Discussion

In this population-based study of men, proinsulin independently predicted death from MI, CHD, or CVD over a period of up to 26.7 years and also when adjustments were made for the confounding effects of smoking, blood pressure, and the LDL/HDL cholesterol ratio and for possible confounding effects of body mass index, fasting blood glucose, and triglycerides. The magnitude of the association between proinsulin and the cardiovascular outcomes also influenced the all-cause mortality. Furthermore, proinsulin showed an association with morbidity caused by MI, CHD, or CVD, whereas the associations between both specific insulin and IRI and these outcomes were not independent of the confounders mentioned above.

The role of IRI as a risk factor for CHD has been controversial.15–18 In a meta-analysis of 12 longitudinal studies,1 the relationship between IRI and CVD was found to be modified by the type of insulin assay used. Inasmuch as PLMs constitute a larger proportion of the IRI after a glucose load than in the fasting state,11 the IRI concentrations after an oral carbohydrate intake may represent the contribution of PLMs to cardiovascular risk better than fasting IRI. In good agreement with this suggestion and our findings, the area under the IRI curve, as opposed to the fasting concentrations of IRI, was significantly related to CHD in a 22-year follow-up of Finnish men.3

The significant relationship between the specific insulin concentrations and the development of CHD disappeared after adjustment for confounders. This result suggests that hyperinsulinemia alone does not increase cardiovascular risk, in agreement with results from the United Kingdom Prospective Diabetes Study.19 This observation further supports that an increase in the concentrations of insulin precursors rather than the plasma insulin concentrations per se underlies the association with CHD.6

It has been suggested that the association between immunoreactive plasma insulin and CVD may have been confounded by comorbidity in earlier cohort studies.20 To circumvent such a possibility in the present study, subjects with CVD or malignant disease at baseline were excluded from our analyses. Because subjects with diabetes, who could be
expected to have elevated plasma concentrations of proinsulin, were included in the study, the multivariate models were adjusted for fasting glucose concentrations. Furthermore, proinsulin has been shown to predict, independently, the development of type 2 diabetes, and type 2 diabetes and glycosylated hemoglobin are associated with mortality of cardiovascular and all causes. Therefore, we performed separate analyses, excluding prevalent and incident diabetic subjects identified during the course of the study, in which the relationships between proinsulin and the defined outcomes remained significant, albeit lower in terms of HRs.

The half-life of proinsulin is considerably longer than that of insulin. Proinsulin is therefore subject to smaller fluctuations, leading to lower intraindividual variation of proinsulin than of insulin measurements. The higher stability of a point estimate of proinsulin, than of specific insulin, may thus contribute to the better predictive capacity of proinsulin for CHD in comparison with specific insulin. This may favor proinsulin in comparison with specific insulin in a regression analysis. Proinsulin may therefore turn out be more appropriate than specific insulin for estimation of future cardiovascular risk.

The mechanisms by which proinsulin, but not specific insulin, may contribute to the progression of CHD are unknown. Cross-sectional angiography studies in nondiabetic subjects who survived their first MI showed that high plasma proinsulin concentrations were associated with more advanced coronary atherosclerosis. Plasma proinsulin has also been found to have stronger associations than insulin with hypertension, dyslipidemia, and impaired glucose tolerance. Fasting proinsulin was found to be more strongly correlated to measurements of insulin resistance than to measurements of acute insulin response, similar to the pattern of fasting specific insulin, whereas the fasting proinsulin to insulin ratio was proposed as a marker for insulin secretion. Thus, rather than being directly associated with its cause, proinsulin may be a marker of an underlying metabolic disturbance predisposing to atherosclerosis.

In vitro, proinsulin increases the plasminogen activator inhibitor-1 (PAI-1) production, and in humans, plasma proinsulin was directly associated with PAI-1 concentrations. PAI-1, as a major inhibitor of fibrinolysis, has the capacity of constituting an important factor in the pathogenesis of atherothrombosis preceding the onset of ischemic heart disease by several years at the population level. A lag phase of 5 years between the elevated plasma proinsulin and the increased risk of CHD (Figure 2) may explain why 2 shorter studies failed to reveal the association.

The few clinical trials comparing human proinsulin and insulin treatment in diabetic subjects have shown that the overall frequency of cardiovascular events was 7 to 18 times higher when human proinsulin was administered for 1 to 2 years, compared with that of human insulin. The attained plasma proinsulin concentrations were clearly supraphysiological, but on the other hand, the higher cardiovascular event rate became apparent after a comparatively short time period.

We conclude that proinsulin is a strong and statistically highly significant predictor of coronary heart disease independent of the other major risk factors smoking, elevated blood pressure, and serum cholesterol concentrations. The increased risk for CHD associated with proinsulin seems to be restricted to the third of the population with the highest proinsulin concentrations.

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References


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