High Proinsulin Concentration Precedes Acute Myocardial Infarction in a Nondiabetic Population

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Hyperinsulinemia has been shown to have strong and consistent associations with a cluster of cardiovascular risk factors. Yet the associations between hyperinsulinemia and coronary heart disease (CHD) have been weak, at best, and often inconsistent. Most previous studies have analyzed the insulin level using a radioimmunoassay method, which does not separate proinsulin from intact (true) insulin. New methods separating the two have demonstrated that proinsulin may be at least as strongly or even more strongly associated than intact insulin with a CHD-promoting risk factor profile. In this incident case-control study of a nondiabetic population, 67 cases of first acute myocardial infarction (AMI) were compared with 127 individually age- and sex-matched controls. Blood sampling was collected prior to disease outcome. Proinsulin and intact insulin levels were measured using highly sensitive two-site sandwich enzyme-linked immunosorbent assays (ELISAs). The highest quartile of proinsulin, in contrast to intact insulin, showed a greater than threefold increase in AMI compared with the lowest quartile, with an odds ratio (OR) and 95% confidence interval (CI) of 3.5 and 1.2 to 9.9, respectively. The increased risk of AMI persisted after controlling for total cholesterol, smoking status, diastolic blood pressure, and antihypertensive medication, and disappeared after additional control was used for the body mass index. High levels of proinsulin, even in a nondiabetic population, seem to be a strong and independent risk factor for AMI. The mechanism underlying the relationship may be direct via effects on fibrinolysis or, probably more plausibly, indirect, where proinsulin is a marker of an underlying metabolic disturbance.

DURING THE PAST DECADE, ample evidence has been presented on the association between the insulin level and a cluster of cardiovascular risk factors. The risk factors for the insulin resistance syndrome are obesity (especially of the upper body), dyslipidemia with high triglyceride and low high-density lipoprotein (HDL) cholesterol levels, hypertension, and low fibrinolytic activity. The syndrome sometimes also includes impaired glucose tolerance (IGT).[1-3]

The epidemiologic evidence from prospective studies on the relations between the insulin level and coronary heart disease (CHD) has not been convincing.[4,5] In some studies, fasting insulin,[6] and in others stimulated insulin,[7] has been associated with CHD. The relations have been found in men but not in women,[8] and sometimes early in the study but not at a later follow-up date.[9] Perhaps there are natural explanations for these inconsistent results in the differences between studies in terms of sample size, population, and methodology.[10] Yet another possible explanation may be that it is not the elevated plasma insulin level per se, but an increase in its precursors proinsulin and split proinsulin, that constitutes the association with CHD.[4]

Most previous studies have used insulin assays that measure total immunoreactive insulin, which largely includes proinsulin and its conversion intermediates. The introduction of specific immunoradiometric[11] or enzyme-linked immunosorbent (ELISA) assays[12,13] that separate proinsulin and split proinsulin from intact (true) insulin made it possible to examine independently the effect of proinsulin and intact insulin on cardiovascular risk factors and CHD. It has already been shown in both diabetics and nondiabetics that proinsulin and split proinsulin have a stronger association than insulin with dyslipidemia (high triglycerides and low HDL-cholesterol), hypertension, and IGT.[14] It also has been shown that proinsulin increases plasminogen activator inhibitor type 1 (PAI-1) activity and thereby decreases fibrinolytic activity.[15,16] This agrees with the finding that insulin therapy in type 2 diabetic patients decreased both the proinsulin level and PAI-1 activity.[17] Furthermore, a clinical trial treating type 2 diabetic patients with human proinsulin was terminated prematurely because of a cluster of myocardial infarctions in the treated group.[18] Recently, high proinsulin levels were shown to be associated with more severe coronary atherosclerosis in young nondiabetic male survivors of acute myocardial infarction (AMI).[19]

The aim of the present study was to evaluate, within an incident case-control study of a nondiabetic population, proinsulin and intact insulin as risk factors for a first myocardial infarction.

SUBJECTS AND METHODS

Study Population

Since 1985 in the province of Västerbotten in northern Sweden, there has been an ongoing community intervention program on cardiovascular disease and diabetes—the Västerbotten Intervention Program (VIP). As part of this program, all men and women were invited to a health survey at the age of 30, 40, 50, and 60 years. At the same time, the two most northern provinces of Sweden, Vasterbotten and Norrbotten, joined the World Health Organization (WHO) Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) Study,[20] wherein a similar health survey was made in 1986, 1990, and 1994 using randomly selected participants aged 25 to 64 years. In both the VIP and the MONICA health surveys, participants were requested to donate a

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blood sample to be stored at the Northern Sweden Medical Research Bank for future research purposes.

In the present study, a nested case-control design was used, with incident cases defined by the Northern Sweden MONICA Incidence Registry.[21] The inclusion criteria for case status were a registered first AMI during the period from January 1985 to September 1994 combined with participation (including a blood sample donation) in the VIP or MONICA health surveys prior to the AMI. Individuals were excluded if they had a cancer diagnosis or if the blood sample was inadequate for analysis. At the time cases were defined (September 30, 1994), about 36,000 persons had participated in the VIP or MONICA health surveys. More than 91% of the participants donated blood samples to the Northern Sweden Medical Research Bank. Two controls for every case were randomly selected among the participants. They were matched for sex, age (±2 years), date of health survey (±1 year), and geographical region. The controls were excluded if they died, moved out of the region, or had a cancer diagnosis. They were also excluded if they reported a prior AMI or stroke to the Northern Sweden MONICA Incidence Registry or on the health survey questionnaire, or if AMI or stroke prior to the health survey could not be excluded from the case record. For the present study, four individuals (one case and three controls) were excluded because of missing values for the main variables (proinsulin and intact insulin). In addition, all individuals with known (n = 7) or unknown (n = 8) diabetes at the health survey were excluded. An oral glucose tolerance test (OGTT) was performed in 90% (174 of 194) of the participants.[22] A fasting plasma glucose level of 7 mmol/L or higher[23] or a 2-hour plasma glucose level in the diabetic range during an OGTT were used as the criteria for diabetes. After exclusion of 10 cases and five controls with diabetes along with exclusion of the individually matched controls for the diabetic cases (n = 21), 67 cases (55 men and 12 women) and 127 controls (104 men and 23 women) remained and formed the basis of the present study.

Methods

At the health survey, blood pressure was measured after 5 minutes' rest. Body weight was measured in light indoor clothing and recorded to the nearest kilogram. Height was measured to the nearest centimeter without shoes. The body mass index was calculated as weight (kilograms) divided by height (meters) squared. Smoking habits were classified in two different ways. Smokers were defined as those reporting daily smoking of cigarettes, cigarillos, cigars, or a pipe. One of the smoking variables classified exsmokers and occasional smokers as nonsmokers. For the other smoking variable, exsmokers and occasional smokers were classified separately. During the first years of the VIP health surveys, the minimum fasting period before blood sampling was 4 hours. Since most of the health surveys were performed in the morning, a majority of the participants had an overnight fast. From 1992, the requested minimum fasting period was changed to 8 hours. In a majority of the subjects (n = 174), an abbreviated OGTT was performed according to the WHO standard with a 75-g anhydric glucose load and measurement of plasma glucose after 2 hours.[22] Venous blood was sampled in heparin tubes to obtain plasma for analyses of proinsulin and intact insulin, and in EDTA tubes for analysis of apolipoprotein A1. The plasma samples were snap-frozen within 1 hour at −20°C, and later on the same day or at least within 1 week, they were stored at −80°C. Fresh plasma was used for measurement of total cholesterol and fasting and 2-hour glucose levels.

Laboratory Procedures

The proinsulin level was measured using a highly sensitive two-site sandwich ELISA.[12] The assay is based on two monoclonal antibodies, a mouse anti–human C-peptide antibody (PEP-001) and a mouse anti–human insulin antibody (HUI-001). The detection limit in human serum is 0.25 pmol/L. There was no cross-reactivity with human insulin and human C-peptide. However, the four major proinsulin conversion intermediates reacted in various proportions of 65% to 99%. The intact insulin level was measured in a similar manner using another sensitive two-site sandwich ELISA.[13] The assay is based on one monoclonal antibody with its epitope near the C-terminal end of the B-chain (OXI-005) and one monoclonal antibody with its epitope centered around the A-chain loop (HUI-018). The detection limit is 5.0 pmol/L. The specificity of the assay excludes intact, split(32-33), and des(31,32) proinsulin. There was some cross-reactivity with split(65-66) proinsulin (30%) and des(64,65) proinsulin (63%). The apolipoprotein A1 level was measured with a commercial radioimmunoassay research kit (RIA-100; Kabi Pharmacia, Uppsala, Sweden). Total serum cholesterol, fasting plasma glucose, and 2-hour plasma glucose concentrations were measured in the VIP health surveys using Reflotron bench-top analyzers (Boehringer, Mannheim, Germany) at the time of the health survey.[24]

The study was approved by the Research Ethics Committee at Umeå University, and the data-handling procedures were approved by the National Computer Data Inspection Board.

Statistical Analysis

The Statistical Analysis System (SAS) version 6.12 (SAS Institute, Cary, NC) and EGRET software version 1.01.10 (SERC, Seattle, WA) were used. Our main variables (proinsulin and intact insulin) had a skewed distribution. An approximate normal distribution was achieved after logarithmic transformation, and all statistical analyses on these variables were performed on transformed values. The sample was divided into quartiles of proinsulin, intact insulin, and total cholesterol, defined by the distribution of these variables among the controls. This procedure was repeated for the group that underwent the OGTT and for the group with normal glucose tolerance. Univariate conditional logistic regression analyses were performed to estimate the odds ratio (OR) and 95% confidence interval (CI) for a first myocardial infarction for different levels of proinsulin, intact insulin, and cholesterol. Multiple conditional logistic regression analysis was performed on the association between myocardial infarction and proinsulin after adjustment for other cardiovascular risk factors. Thirteen subjects had missing values for the smoking variable, and three subjects had missing values for the body mass index and blood pressure. In the conditional logistic regression analyses, missing values for continuous variables were replaced by the mean value for the control group, whereas missing values for categorical variables were treated as a separate category.

RESULTS

The geometric mean and 95% CI for our main variables, proinsulin and intact insulin, are listed separately for men and women in Fig 1. There were only 12 female cases in our study population. In men, the mean level of proinsulin was 8.7 pmol/L among cases and 7.3 pmol/L among controls. The corresponding values in women were 8.3 versus 6.0 pmol/L. In men, the mean level of intact insulin was 28.5 pmol/L among cases and 22.3 pmol/L among controls, as compared with 37.7 versus 25.2 pmol/L in women. In both genders and for both proinsulin and intact insulin, cases showed higher levels than controls. This together with the low number of female cases supported the decision to present only the merged results for men and women.

The baseline clinical and laboratory characteristics of cases and controls are shown in Table 1. There was no difference in age between cases and controls. The mean levels of proinsulin ($P = .07$) and intact insulin were higher among cases versus controls. The same result was found also for the total cholesterol, systolic and diastolic blood pressure, body mass index ($P = .06$), and proportion of smokers. Apolipoprotein A1 was

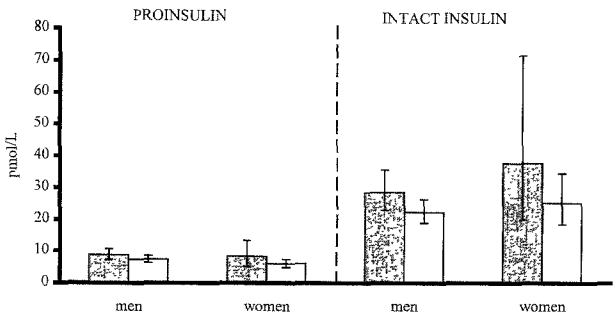

Fig 1. Geometric mean and 95% CI for proinsulin and intact insulin in cases (■) and controls (□) presented separately for men and women.

significantly lower in cases than in controls. There was a high correlation between the level of proinsulin and intact insulin. There was also a high correlation between our insulin measures and the body mass index (Table 2).

Ninety percent of the study group (n = 174) underwent an OGTT. Twelve of these (7%) fulfilled criteria for IGT. IGT subjects were older (59.5 *v* 53.5 years, $P < .01$) and had a higher age-adjusted body mass index (29.6 *v* 25.8 kg/m^2, $P < .001$) and higher age-adjusted proinsulin level (13.6 *v* 7.3 pmol/L, $P < .01$) than the subgroup with normal glucose tolerance. Nine of 12 IGT subjects (three cases and six controls) were classified in the highest quartile of proinsulin.

Figure 2 presents the crude OR for a first myocardial infarction when exposed to different levels of proinsulin, intact insulin, or total cholesterol. The relative risk of a myocardial infarction increased with increasing levels of proinsulin. The OR (with 95% CI) for quartiles two to four of proinsulin was 3.0 (1.1 to 8.4), 2.6 (1.0 to 7.2), and 3.5 (1.2 to 9.9), respectively. The corresponding OR for intact insulin was 1.3 (0.5 to 3.2), 1.5 (0.6 to 3.8), and 1.6 (0.7 to 4.0), respectively. The OR for myocardial infarction when exposed to different quartiles of total cholesterol showed a U-shaped configuration. There was a decrease in the OR, although nonsignificant, when the second quartile of cholesterol was compared with the first with an OR (95% CI) of 0.5 (0.2 to 1.5). Thereafter, the OR increased with increasing cholesterol levels to 1.1 (0.5 to 2.7) in the third and 2.1 (0.9 to 4.8) in the fourth quartile.

Table 1. Baseline Characteristics of Cases and Controls

Characteristic	Cases (n = 67)	Controls (n = 127)	*P*
Age (yr)	54 4	54.2	
Sex (male/female)	55/12	104/23	
Proinsulin (pmol/L)*	8.6	7.1	.073
Intact insulin (pmol/L)*	29.9	22.8	.033
Body mass index (kg/m^2)	26.7	25 6	.063
Systolic blood pressure (mm Hg)	142.8	136.3	.051
Diastolic blood pressure (mm Hg)	87.9	84.4	.046
Total cholesterol (mmol/L)	6.81	6.33	.014
Apolipoprotein A1 (mg/L)	1,088	1,154	.011
Fasting plasma glucose (mmol/L)†	5.1	5.1	.926
2-hour plasma glucose (mmol/)†	6.2	6.2	.968
Daily smoking (%)‡	48	30	.020

*Geometric mean.
†n = 174.
‡Chi-square test

Table 2. Pearson Correlation Coefficients for Proinsulin and Intact Insulin

Variable	Proinsulin	Intact Insulin
Proinsulin	1.00	
Intact insulin	.73‡	1.00
Body mass index	.58‡	.55‡
Systolic blood pressure	.25‡	.16*
Diastolic blood pressure	.31‡	.22†
Total cholesterol	.15*	.24‡
Apo lipoprotein A1	−.11	−.05
Sample storage time§	.01	−.09

*$P < .05$.
†$P < .01$.
‡$P < .001$.
§Difference between the stop date of the study and the date of the health survey.

In Table 3, the association between the proinsulin level and risk of myocardial infarction is presented before and after controlling for potential confounders. In this analysis, total cholesterol and diastolic blood pressure were used as continuous variables. Smoking habits were classified in two different ways. There was a minor but nonsignificant increase in the OR point estimates and CIs when the smoking variable "daily smoker/nonsmoker" was replaced with the more precise smoking variable, "daily smoker/exsmoker/occasional smoker/nonsmoker." The adjusted OR for the second, third, and fourth quartiles of proinsulin was 3.1 (1.0 to 9.7), 2.0 (0.6 to 6.6), and 3.5 (1.1 to 11.6), respectively. Additional adjustment for the body mass index decreased the OR mainly in the fourth quartile, 3.1 (0.9 to 11.2).

Conditional logistic regression analyses before and after adjustment for potential confounders were also performed on the subset of the study group that underwent an OGTT (n = 174). Of these, 164 subjects (58 cases and 106 controls) were eligible for the analyses. The OR point estimates were generally somewhat higher and the CIs somewhat larger than in the whole study group. The only significant difference between the two groups was that the OGTT group, after controlling for potential confounders including the body mass index, still had

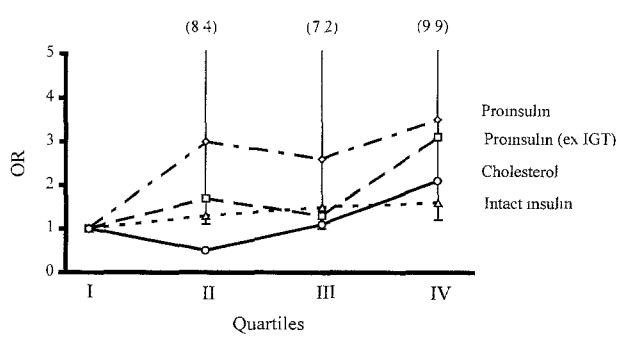

Fig 2. OR for first myocardial infarction ordered according to increasing levels of proinsulin, intact insulin, and total cholesterol, respectively. In addition, ORs for proinsulin in the subgroup with normal glucose tolerance are presented (proinsulin ex IGT). The first quartiles were used as reference levels. For proinsulin, 95% CI is presented. The OR and CI were calculated using univariate conditional logistic regression analysis.

Table 3. OR and 95% CI for First Myocardial Infarction in Different Quartiles of Proinsulin Before and After Adjustment for Potential Confounders for the Whole Study Group and the Subgroup with Normal Glucose Tolerance

Quartile	No. of Subjects		Crude Proinsulin		Adjusted Proinsulin*		Adjusted Proinsulin†	
	Cases	Controls	OR	95% CI	OR	95% CI	OR	95% CI
All subjects (N = 194)								
I (<4.4 pmol/L)	8	34	1.0		1.0		1.0	
II (4.4-6.3 pmol/L)	20	32	3.0	1.1-8.4	3.1	1.0-9.7	3.0	1.0-9.6
III (6.4-9.9 pmol/L)	18	31	2.6	1.0-7.2	2.0	0.6-6.6	2.0	0.6-6.5
IV (>9.9 pmol/L)	21	30	3.5	1.2-9.9	3.5	1.1-11.6	3.1	0.9-11.2
Normal glucose tolerance (n = 142)								
I (<4.3 pmol/L)	7	21	1.0		1.0		1.0	
II (4.3-5.9 pmol/L)	13	24	1.7	0.6-5.1	1.7	0.5-5.7	1.6	0.5-5.7
III (6.0-7.8 pmol/L)	9	22	1.3	0.4-4.4	1.3	0.3-5.7	1.3	0.3-5.7
IV (>7.8 pmol/L)	23	23	3.1	1.1-9.0	2.8	0.8-10.3	3.4	0.9-13.6

*Adjusted for total cholesterol, diastolic blood pressure, antihypertensive medication, and smoking.
†Adjusted for the above variables plus body mass index.

an increased risk for myocardial infarction with an OR (95% CI) of 4.5 (1.2 to 16.8) when the highest quartile of proinsulin was compared with the lowest. Additional control for fasting plasma glucose or 2-hour plasma glucose did not change the result.

The OR for a myocardial infarction at different levels of proinsulin was calculated in a similar manner in the subgroup with normal glucose tolerance, after exclusion of those who did not undergo an OGTT (n = 20) and those who fulfilled criteria for IGT (n = 12). In the conditional logistic regression analyses, 142 subjects (52 cases and 90 controls) were eligible, and classification of proinsulin into quartiles was based on the distribution of the control values (n = 90) in the data set. Figure 2 (proinsulin ex IGT) and Table 3 show that the crude OR was significant only in the fourth quartile, 3.1 (1.1 to 9.0). After adjusting for potential confounders, there was no difference in the OR between different levels of proinsulin.

DISCUSSION

The present study establishes proinsulin as an independent risk factor for first myocardial infarction. Increasing levels of proinsulin, in contrast to increasing levels of intact insulin and total cholesterol, increased the risk of myocardial infarction in the univariate analyses (Fig 2). The mean level of proinsulin among controls in this study (7.1 pmol/L) resembles that of a healthy population from the same area of Sweden (6.9 pmol/L),[25] suggesting that the controls of this study, on average, have a normal level of proinsulin. Inclusion in the highest quartile of proinsulin with levels higher than 9.8 pmol/L, compared with the lowest quartile with levels less than 4.4 pmol/L, was associated with a greater than threefold increase in the risk of myocardial infarction. The risk factor status of proinsulin for myocardial infarction was still present after controlling for the traditional cardiovascular risk factors of total cholesterol, smoking status, diastolic blood pressure, and antihypertensive medication. However, it disappeared after additional control for the body mass index (Table 3). It seems that most of the increased risk attributed to proinsulin in the present nondiabetic population may be explained by the subjects' having IGT and/or the insulin resistance syndrome.[19,26]

In the present study, the ORs for myocardial infarction associated with different levels of intact insulin were only half of the ORs for proinsulin (Fig 2). However, our study and other studies[25,27] show that intact insulin increases some risk factor variables and worsens the total cardiovascular risk factor profile. The mechanisms by which proinsulin and possibly intact insulin may cause ischemic heart disease are incompletely understood, and conflicting results have been recently published.[28,29] One leading hypothesis focuses on the role of hemostasis in general and especially on the associations with disturbed fibrinolysis. In experimental studies with both endothelial and liver cells, proinsulin has been shown to increase PAI-1 activity.[15,16] Insulin therapy in type 2 diabetic patients has been found to suppress both PAI-1 activity and proinsulin secretion.[17] Furthermore, in patients with coronary artery disease, strong associations have been found repeatedly between the proinsulin level and PAI-1 activity.[30,31]

Another possible explanation for the relation between proinsulin and first myocardial infarction is that the level of proinsulin, which reflects the actual demand on β cells,[32] functions as a sensitive marker of an underlying metabolic disturbance.[33] In this respect, proinsulin seemed independent of the glucose level, since the association remained between proinsulin and myocardial infarction after adjustment for fasting or 2-hour plasma glucose.

One reason for the unexpectedly low ORs of increasing cholesterol levels on myocardial infarction was the finding of twice as many cases in the first quartile of cholesterol (n = 15) as in the first quartile of proinsulin (n = 8). Of course, this could be due to chance. Another reason might be the fact that in northern Sweden the entire population, on average, has a high total cholesterol concentration, manifested here as a small difference in cholesterol between cases and controls, 6.8 versus 6.3 mmol/L. Probably, the relative importance of other cardiovascular risk factors will increase in such a population.

It is important to emphasize the incident case-control design of this study, implying that the exposure factors were measured before disease developed. This excludes the possibility that high proinsulin levels were an effect of previous myocardial infarction.

The change in the minimum fasting time before blood sampling from 4 to 8 hours that was introduced in the VIP health

surveys from 1992 does not seem to pose any problem with respect to the findings in this study between proinsulin and acute myocardial infarction. The group with a minimum of 4 hours of fasting showed nonsignificantly higher mean values for proinsulin in both cases and controls compared with the group with a minimum of 8 hours of fasting. The possible imprecision in the classification of exposure status introduced by suboptimal fasting conditions must have applied at random to cases and controls, since the exposure factors were collected prior to disease outcome. Nondifferential misclassification of exposure always results in underestimation of the true relative risk.[34]

It is not easy to compare the impact of different cardiovascular risk factors in the etiology of myocardial infarction. The discussion as to whether insulin (proinsulin and/or intact insulin) is an independent risk factor for myocardial infarction is obscure. The etiology of myocardial infarctions is said to be multifactorial, which means that there are several different causative mechanisms leading to the disease. In this mosaic of more or less related risk factors, some will be joined in the same causal path while others will be joined in another causal path.[34] By defining a risk factor as independent, we must mean that it is independent of other causal paths. However, we often do not know the exact relations between our measured risk factor variables and the causal path to which they belong. In our study, the body mass index and proinsulin were highly correlated, and it is certainly biologically plausible that these two variables act in the same causal path in the etiology of myocardial infarction. The association between myocardial infarction and proinsulin was also reduced when the body mass index was introduced into the regression model. The use of multiple regression analyses emphasizes the need for careful interpretation.

In conclusion, this incident case-control study, in which sampling occurred before disease outcome, predicted a greater than threefold increase in first myocardial infarction in nondiabetic individuals with high proinsulin concentrations.

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