Are Insulin and Proinsulin Independent Risk Markers for Premature Coronary Artery Disease?

Richard J. Katz, Robert E. Ratner, Robert M. Cohen, Ellen Eisenhower, and Dante Verme

Controversy persists about whether hyperinsulinemia and hyperproinsulinemia are independent risk markers for coronary atherosclerosis. A common limitation of most previous studies has been imprecise categorization of disease status in normal and coronary artery disease (CAD) groups. We assessed the relationship of pancreatic β-cell secretory products and premature CAD in a case-control study of 134 nondiabetic subjects, aged ≤55 years old, carefully defined for CAD status by catheterization and/or thallium stress studies. Case patients comprised 66 patients with premature CAD, and control subjects (non-CAD group) included 68 patients without CAD but with traditional CAD risk factors and chest pain and/or abnormal electrocardiograms but normal catheterization and/or thallium stress studies. In addition to the CAD and non-CAD group comparison, both groups were compared with a reference group of 27 mixed lean and obese control volunteers. All CAD and non-CAD patients had a 3-h 75-g oral glucose tolerance test with measurement of fasting and post–glucose load immunoreactive insulin (IRI), specific insulin (INS), proinsulin-like material (PI), and C-peptide. Increased fasting insulin and fasting proinsulin levels both were statistically significantly associated with higher odds of being in either the premature CAD and the non-CAD groups when compared with the reference group in a polychotomous logistic regression model (odds ratio of at least 1.20 for a 20% increase in each β-cell secretory product in both comparisons, $P < 0.05$). However, increased pancreatic β-cell secretory hormone levels did not show a statistically significant relative risk for being in the premature CAD group when compared with the non-CAD group. After adjustment for BMI, all statistically significant associations disappeared for IRI, INS, and PI when the odds favoring being in the CAD and non-CAD groups were compared versus the reference group. Furthermore, the odds of being in the premature CAD and non-CAD groups when compared with the reference group were not significantly associated to the ratio of PI to insulin and C-peptide. Thus, although there is a statistically significant association between the odds of having premature CAD with elevated insulin and proinsulin levels compared with the reference group, these findings are equally common in subjects with traditional CAD risk factors without detectable CAD. Furthermore, the association of higher insulin and proinsulin levels with the likelihood of a patient having or not having CAD disappears after adjustment for BMI, suggesting that insulin and proinsulin are not independent risk markers but are primarily dependent on obesity. *Diabetes* 45:736–741, 1996

In recent years, hyperinsulinemia has been associated with coronary artery disease (CAD) in both retrospective and prospective studies (1–3). Despite 20 years of research, however, the strength of this relationship remains the subject of intense debate (4–8). In epidemiological studies, the association of insulin with CAD has been inconsistent and, at times, weak at best. Results differ depending on the analysis (univariable and multivariable) and endpoints (fasting versus post–glucose load insulin). Each of these studies has had limitations that may account for the conflicting results. Commonly, the population studies included CAD patients and normal subjects without strict documentation of the disease status. This has been the case particularly for the control subjects, who usually comprise an age-matched cohort without known clinical manifestation of atherosclerosis. Only a few retrospective reports include patients specifically categorized by noninvasive (stress testing) or invasive (angiographic) testing (9–11). Additionally, most reports have used total insulin immunoreactivity, which reflects insulin and variable percentages of proinsulin and intermediates in the conversion of proinsulin to insulin. This has raised questions about the role of proinsulin in the relationship of total insulin immunoreactivity to CAD. Assays specific for and distinguishing between insulin and proinsulin have been used only in a few of the epidemiological and none of the angiographic studies.

Some investigators suggested that whereas insulin may not be an independent risk marker for CAD, it is the hyperinsulinemic syndrome, a cluster of factors, that results in atherosclerosis (6,7). This syndrome comprises hyperinsulinemia, hypertension, NIDDM, and central obesity. Among these markers, however, it may be android obesity, rather than insulin, that is central to CAD risk. Another theory has promoted proinsulin, rather than insulin, as a primary factor for CAD risk (12,13). If proinsulin is significant, it may be only in patients with frank diabetes who commonly have proinsulin secretion disproportionate to that of insulin (14,15). In nondiabetic subjects, however, proinsulin concentration usually parallels insulin concentration, and thus CAD risk should be equally strong or weak for both hormones. The importance of proinsulin deserves further study, since

From the Department of Medicine (R.J.K., E.E., D.V.), Division of Cardiology, George Washington University School of Medicine; the Medlantic Research Institute (R.E.R.), Washington, DC; and the Department of Medicine (R.M.C.), Division of Endocrinology/Metabolism and General Clinical Research Center, University of Cincinnati College of Medicine, Cincinnati, Ohio.

Address correspondence and reprint requests to Dr. Richard J. Katz, Division of Cardiology, George Washington University Medical Center, 2150 Pennsylvania Ave., N.W., Washington, DC 20037.

Received for publication 8 June 1995 and accepted in revised form 18 January 1996.

AUC, area under the curve; CAD, coronary artery disease; ECG, electrocardiogram; IGT, impaired glucose tolerance; INS, specific insulin; IRI, immunoreactive insulin; PI, proinsulin-like material; WHR, waist-to-hip ratio.

one recent study by Haffner et al. (16) found relative increased proinsulin to insulin levels in nondiabetic subjects with the insulin resistance syndrome.

The aim of the present study was to evaluate the relationship of pancreatic β-cell secretory products to premature CAD in a population carefully defined for CAD status. All patients had undergone either cardiac catheterization or exercise thallium imaging with measurement of specific insulin and proinsulin.

RESEARCH DESIGN AND METHODS

Subjects. Study subjects undergoing treatment and evaluation in the coronary care unit, exercise laboratory, and cardiac catheterization laboratory at the George Washington University Medical Center were asked to participate. All individuals were ≤55 years old. None had diabetes, as determined by glucose tolerance testing (see below). Sampling was designed to balance sex and race (blacks and whites) in the CAD group and the group without CAD (non-CAD group). The presence of CAD was determined by coronary arteriography (>50% stenosis in one or more major coronary vessel) in all patients in whom CAD was positively confirmed. The absence of CAD was documented by either a negative exercise thallium perfusion study and/or a normal cardiac catheterization (no lesions). For comparison with the non-CAD and CAD patient groups, we used a reference group of 27 mixed lean and obese volunteer subjects studied at the University of Cincinnati. Studies were approved by the Committees on Human Research at the George Washington University Medical Center and the University of Cincinnati, and written signed consent was obtained from each participant.

Data collection. Physical characteristics measured included BMI, waist-to-hip ratio (WHR), and percentage body fat. Waist and hip circumferences were measured according to standard procedures. The waist was measured to the nearest centimeter at the level of the umbilicus with the subject standing. Hip circumference was measured at the level of the iliac crest with the subject in the supine position. Bioelectrical impedance analysis was used for assessment of body composition. Measurements were obtained using a four-electrode impedance plethysmograph (RJL, Detroit, MI). The analyzer takes into account the subject's height and total body weight and estimates percentages of lean and fat body mass.

Glucose tolerance testing. All subjects had an oral glucose tolerance test performed after an overnight 12-h fast. For the test, 75 mg oral glucose was administered at 9:00 A.M. Blood samples were drawn at −20, −10, 0, 15, 30, 45, 60, 90, 120, and 180 min for glucose and pancreatic β-cell secretory products.

Laboratory analyses. Plasma glucose was measured using a glucose oxidase method (Glucose Analyzer II, Beckman, Brea, CA). Insulin, proinsulin, and C-peptide were measured in the laboratory of Dr. R. Cohen. Insulin was measured using two equilibrium radioimmunoassays with second-antibody precipitation (17): one for immunoreactive insulin (IRI) and one specific for insulin (INS). The IRI assay is based on an antiserum with ~50% cross-reactivity for proinsulin and proinsulin conversion intermediates. The INS assay (Linco, St. Louis, MO) is based on an antiserum with <1% cross-reactivity for proinsulin and des-31,32-proinsulin but ~100% cross-reactivity for des-64,65-proinsulin. Since there are negligible amounts of des-64,65-proinsulin in the circulation, this assay is essentially a measure of insulin itself without significant cross-reactivity from proinsulin-related peptides. Proinsulin-like material (PI) was measured using antiserum 11E in a nonequilibrium assay with second-antibody precipitation, as previously described (18, 19). C-peptide was measured using antiserum M1230 in an alcohol precipitation nonequilibrium assay (20). Intra- and interassay limits of detection were 5 and 8% for IRI, 6 and 8% for INS, 7 and 8% for PI, and 5 and 6% for C-peptide, respectively. Intra- and interassay coefficients of variation were 8 and 7% for IRI, 9 and 9% for INS, 9 and 6% for PI, and 2 and 7% for C-peptide, respectively. The reference range established in the laboratory was based on a sample of 27 nondiabetic mixed lean and obese subjects (12 men and 15 women with BMI of 25 ± 4 [mean ± SE]).

Three ratios were calculated to relate proinsulin to insulin: *1*) proinsulin/(specific insulin plus proinsulin), *2*) proinsulin/C-peptide, and *3*) proinsulin/specific insulin. The glucose response to an oral glucose load was defined as normal, impaired glucose tolerance (IGT), and diabetes by World Health Organization criteria (21). Excluded from analysis were patients with previously known diabetes or an abnormal oral glucose tolerance test that met the criteria for diabetes. Values reported for glucose and pancreatic β-cell secretory products were baseline, 2 h, peak, and area under the curve (AUC).

TABLE 1
Demographic and clinical characteristics of the study population

	Non-CAD group	CAD group	*P* value
Demographics			
n	68	66	
Age (years)	44 ± 0.8	47 ± 0.8	0.011
Ethnicity (% blacks)	58	44	0.125
CAD risk factors			
Male sex (%)	47	54	0.409
History of smoking (%)	50	68	0.039
History of hypertension (%)	69	81	0.133
History of dyslipidemia (%)	64	61	0.763
Family history of CAD (%)	55	44	0.310
Metabolic profile			
Family history of diabetes (%)	23	18	0.476
Abnormal glucose tolerance test (% IGT)	25	28	0.716
Body fat (%)	35 ± 1.2	30 ± 1.4	0.011
BMI (kg/m^2)	31 ± 0.8	29 ± 0.8	0.113
WHR	0.86 ± 0.01	0.88 ± 0.01	0.222
Fasting glucose (mg/dl)	97 ± 1.8	98 ± 1.9	0.813

Data are means ± SE.

Statistical analysis. The data were analyzed using PC SAS for Windows software. Statistical comparison of the prevalence of nominal traditional risk factors between the CAD and non-CAD groups was conducted using the χ^2 test statistic. The statistical significance of continuous traditional risk factors with the risk of premature CAD was determined using Wald's χ^2 test statistic obtained from a bivariable logistic regression analysis.

Wald's χ^2 test statistic from a bivariable logistic regression analysis was also used to determine if there was a statistically significant association between the natural logarithm of each β-cell secretory product and product ratios with the odds of having premature CAD. The above analyses were conducted separately on baseline, 2-h, peak, and AUC values. AUC curve values were calculated using the standard trapezoidal rule.

The statistical association of the β-cell secretory products with the inclusion of the reference group, in addition to the non-CAD and CAD groups, was determined using a multiple polychotomous logistic regression model. For all statistical comparisons, $P < 0.05$ was considered to indicate a statistically significant association.

RESULTS

Demographics, major CAD risk factors, and metabolic profile. Descriptors of the study population are listed in Table 1. The non-CAD group was slightly younger than the group with premature CAD, but both groups had mean ages in the mid-40s. The recruitment strategy was successful in achieving a balance between men and women and blacks and whites.

The prevalence of major CAD risk factors (male sex, smoking, hypertension, dyslipidemia, and family history of CAD) was comparable in both groups. The high frequency of risks in the non-CAD group reflects the screening process, which included patients with suspicion of CAD who had been referred for either thallium exercise or catheterization tests. None had diabetes, since this was an exclusionary criterion for study participation.

Both the non-CAD and the premature CAD groups were obese and had BMIs of 31 ± 0.8 and 29 ± 0.8 (mean ± SE), respectively. WHRs were similar in both groups (0.86 ± 0.01 in the non-CAD group and 0.88 ± 0.01 in the CAD group). Percentage body fat was slightly higher in the non-CAD group (35 vs. 30% in CAD patients). Although fasting glucose

TABLE 2
Fasting pancreatic β-cell secretory products

				Logistic regression analysis results					
				Non-CAD vs. reference		CAD vs. reference		CAD vs. non-CAD	
	Reference	Non-CAD group	CAD group	Unadjusted	BMI-adjusted	Unadjusted	BMI-adjusted	Unadjusted	BMI-adjusted
IRI (pmol/l)	50 ± 5.8	80 ± 6.4	78 ± 6.9	1.23 (0.005)	1.00 (0.99)	1.21 (0.007)	1.08 (0.44)	0.99 (0.86)	1.08 (0.28)
INS (pmol/l)	50 ± 7.9	76 ± 5.3	77 ± 6.8	1.31 (0.005)	1.16 (0.17)	1.29 (0.007)	1.22 (0.06)	0.99 (0.81)	1.05 (0.49)
PI (pmol/l)	8.6 ± 0.64	13.5 ± 1.39	14.3 ± 1.42	1.20 (0.032)	0.96 (0.74)	1.26 (0.006)	1.09 (0.41)	1.06 (0.36)	1.13 (0.08)
C-peptide (nmol/l)	0.46 ± 0.03	0.68 ± 0.04	0.79 ± 0.05	1.58 (<0.001)	1.22 (0.26)	1.86 (<0.001)	1.73 (0.003)	1.18 (0.07)	1.43 (0.003)

Data are means ± SE or odds ratio (*P*). Odds ratio: the relative odds of being in the first rather than the second group in each comparison for a 20% increase in the pancreatic β-cell secretory product.

was normal in both groups, IGT was frequently seen on glucose tolerance testing. IGT was found in 25% of the non-CAD group and 28% of the CAD group. Despite the high prevalence of glucose handling abnormalities, the incidence of family history of diabetes was low.

β-cell secretory products. Results of the logistic regression analysis of IRI, INS, PI, and C-peptide values are summarized in Tables 2 and 3. The results from all logistic regression analyses are presented as the relative change in odds ratio when the odds of being in the premature CAD group are compared with the odds of being in the non-CAD group for a 20% increase in the corresponding β-cell secretory product or product ratio. The logistic regression analysis was conducted for fasting and postglucose 2-h, peak, and AUC curve values. Pancreatic β-cell secretory products correlated significantly with BMI ($P < 0.0001$ for each β-cell product). Therefore, obesity was assessed as a confounding factor and included as such in a multivariable logistic regression model.

Fasting measures. The levels of insulin and proinsulin were high for both CAD and non-CAD groups. Table 2 presents the statistical results of comparing the odds of being in the reference, non-CAD, or premature CAD groups for a 20% increase in each of the pancreatic β-cell secretory products. The corresponding *P* value is also reported. Patients who had a 20% increase in insulin or proinsulin had a higher chance of being in the premature CAD or non-CAD groups when compared with the laboratory fasting reference control group. However, when the INS and PI values were adjusted for BMI in a multivariable polychotomous logistic regression model, all odds ratios were reduced toward 1.0, and there was no statistically significant difference between any two groups. Only C-peptide yielded significantly higher odds for being in the premature CAD group versus the reference group (odds ratio 1.73, $P = 0.001$).

There was no statistically significant association when the odds of being in the premature CAD group versus the non-CAD group were compared with respect to fasting insulin and proinsulin, with and without adjustment for BMI. C-peptide was the only fasting β-cell product for which the odds of being in the CAD group were higher than being in the non-CAD group (odds ratio 1.43, $P = 0.003$, BMI-adjusted).

Post–glucose load measures. Before adjustment for BMI, there was no statistically significant association between the odds of being in the premature CAD group compared with the non-CAD group with respect to a 20% increase in 2-h, peak, and AUC values for IRI, INS, and PI (Table 3). After adjustment for BMI, only proinsulin values at 2 h and peak were statistically associated with the odds of being in the CAD group compared with the non-CAD group. The estimated odds of being in the CAD group when compared with the non-CAD group for a 20% increase in the 2-h and peak PI values were 1.19 ($P = 0.027$) and 1.17 ($P = 0.041$), respectively. This association was not observed for AUC measures.

TABLE 3
Post–glucose load pancreatic β-cell secretory products

			Logistic regression analysis CAD vs. non-CAD	
	Non-CAD group	CAD group	Unadjusted	BMI-adjusted
2-h				
IRI (pmol/l)	543 ± 44	641 ± 84	1.01 (0.82)	1.06 (0.29)
INS (pmol/l)	488 ± 78	633 ± 58	1.01 (0.78)	1.06 (0.27)
PI (pmol/l)	58.2 ± 5.2	67.1 ± 5.9	1.07 (0.24)	1.19 (0.03)
C-peptide (nmol/l)	2.60 ± 0.15	3.22 ± 0.18	1.27 (0.013)	1.42 (0.003)
Peak				
IRI (pmol/l)	766 ± 74	1,069 ± 210	1.07 (0.17)	1.13 (0.052)
INS (pmol/l)	782 ± 89	942 ± 117	1.05 (0.31)	1.08 (0.22)
PI (pmol/l)	60.1 ± 4.8	71.0 ± 5.8	1.08 (0.19)	1.17 (0.04)
C-peptide (nmol/l)	3.0 ± 0.15	3.57 ± 0.20	1.26 (0.028)	1.35 (0.01)
AUC				
IRI (pmol/l)	79,120 ± 7,731	95,270 ± 12,644	1.04 (0.48)	1.09 (0.16)
INS (pmol/l)	74,470 ± 6,842	84,362 ± 9,126	1.02 (0.74)	1.07 (0.31)
PI (pmol/l)	7,413 ± 673	8,410 ± 673	1.06 (0.35)	1.11 (0.11)
C-peptide (nmol/l)	366 ± 20	445 ± 30	1.18 (0.067)	1.24 (0.03)

Data are means ± SE or odds ratio (*P*). Odds ratio: the relative odds of being in the CAD rather than the non-CAD group for a 20% increase in the post–glucose load pancreatic β-cell secretory product.

TABLE 4
Fasting proinsulin concentration in relation to insulin and C-peptide

				Logistic regression analysis					
				Non-CAD vs. reference		CAD vs. reference		CAD vs. non-CAD	
	Reference group	Non-CAD group	CAD group	Unadjusted	BMI-adjusted	Unadjusted	BMI-adjusted	Unadjusted	BMI-adjusted
PI/INS	25.9 ± 4.0	18.5 ± 1.39	20.7 ± 1.52	0.81 (0.04)	0.84 (0.08)	0.89 (0.26)	0.91 (0.33)	1.10 (0.18)	1.09 (0.26)
PI/(PI + INS)	17.0 ± 0.49	15.1 ± 0.92	16.6 ± 0.93	0.85 (0.19)	0.87 (0.28)	0.96 (0.73)	0.96 (0.78)	1.13 (0.15)	1.11 (0.23)
PI/C-peptide	1.90 ± 0.13	1.93 ± 0.11	1.79 ± 0.11	0.95 (0.59)	0.85 (0.19)	0.91 (0.36)	0.82 (0.11)	0.96 (0.65)	0.97 (0.69)

Data are means ± SE or odds ratio (*P*). Odds ratios compare the relative change in the odds of the two specified groups for a 20% increase in the corresponding ratio.

C-peptide was the only β-cell secretory product that for all post–glucose load measurements was statistically associated with the odds of being in the CAD group compared with the non-CAD group.

Relative proinsulin. Ratios of fasting PI to insulin or PI to C-peptide in plasma are presented in Table 4. There was no consistent statistical association between the ratios when the odds of being in the CAD group versus the non-CAD group were compared, either unadjusted or BMI-adjusted.

DISCUSSION

Twenty-five years after the suggestion that insulin may promote the development of atherosclerosis (22), vigorous debate persists as to the role of pancreatic β-cell products and the atherogenesis of CAD (4,8). The association of hyperinsulinemia with premature atherosclerosis also has been reported in people with and without diabetes (23). The strength of this relationship, however, has been questionable, relying on poorly defined patient groups, a variety of postprandial measures (1–3), and decreased statistical significance after adjustment for obesity. Prospective and retrospective epidemiological studies count ischemic events but do not account for possible silent ischemia in the normal control subjects. In three major prospective clinical studies, significantly higher insulin levels were observed at different times in each study: 1-h postload insulin levels in the Busselton Study (3), 2-h postload insulin levels in the Helsinki Policemen Study (24), and the fasting but not 2-h postload insulin level in the Paris Prospective Study (2).

This study analyzed the relationship of pancreatic β-cell products to CAD status in both men and women ≤55 years old without diabetes, comparing carefully documented patient groups with premature CAD and non-CAD control groups with known traditional CAD risk factors. A 20% increase in insulin or proinsulin was significantly associated with higher odds of being in either the CAD and non-CAD groups when compared with the reference group of 27 mixed lean and obese normal volunteers. The association between β-cell products and the CAD and non-CAD groups compared with the reference group was explained, in part, by obesity. Mean BMI was 29 kg/m^2 in the CAD patients and 31 kg/m^2 in the non-CAD group compared with 26 kg/m^2 in the laboratory reference sample. As in other reports, there was a strong correlation of insulin levels with BMI, both fasting ($P < 0.0001$) and 2-h postload ($P < 0.003$). After adjustment for obesity, the association of pancreatic β-cell secretory products with the odds of being in a particular group disappeared. Additionally, no significant association was noted between INS and IRI, either in the fasting state or after the glucose load, with the odds of having premature CAD compared with patients of comparable age and with similar traditional CAD risks but no detectable CAD. These observations suggest that hyperinsulinemia and hyperproinsulinemia appear to be more a function of a cluster of risk markers than a finding specifically associated with premature CAD.

A 20% increase in C-peptide, which is cosecreted in a 1:1 ratio with insulin, was significantly associated with higher odds of being in the premature CAD and non-CAD groups when compared with the lean laboratory reference group. It was the only β-cell secretory product in this study that was significantly associated with higher odds of being in the CAD group when compared with the non-CAD group, both fasting and after the glucose load. C-peptide was not reported in the prospective Paris, Helsinki, and Busselton studies. Discordance between results for insulin versus C-peptide may reflect differences in hormone distribution and metabolism in vivo (25) or possibly differences in assay performance. The liver removes a large and variable fraction of insulin from portal blood but only negligible amounts of C-peptide. Hepatic extraction of insulin is saturable and thought to be largely insulin receptor–mediated (26). Hence, there may be differences in secretion, which are obscured in the insulin concentrations by variability in distribution space and/or clearance.

Proinsulin has been suggested as an alternative β-cell hormone promoting risk for CAD (12). This hormone commonly is produced in excess in NIDDM subjects (15). Although none of the patients in this study had diabetes, absolute proinsulin concentrations were high in both the CAD and non-CAD groups. But, as with insulin, proinsulin concentrations correlated with BMI ($P < 0.0001$ both fasting and 2-h postload), consistent with obesity-related changes accounting for much of the increase. Hence, while fasting proinsulin concentrations were high, they were not disproportionately increased in relation to either insulin or C-peptide. There was no statistically significant association between any of the ratios of proinsulin to either insulin or C-peptide and the odds of being in the premature CAD group when compared with the non-CAD or reference groups. Because the metabolism of proinsulin is similar to that of C-peptide (27–29), we infer from fasting PI/C-peptide ratios that the ratio of proinsulin secretion to insulin secretion did not significantly differ among these study groups. Likewise, based on the fasting PI(PI + INS) ratio, we do not suspect a statistically significant association of the proportion of proinsulin versus insulin to which most target tissues are exposed with the odds of having premature CAD when compared with being a non-CAD subject. These findings are

in contrast to the report of Haffner et al. (13), who described excess proinsulin in nondiabetic subjects with cardiovascular risk factors. Our study population comprised 50% women and blacks, which may account for some of the interstudy differences. The pathophysiological importance of proinsulin in CAD subjects after a glucose load remains unclear. Proinsulin may contribute to the early onset of clinical atherosclerosis in a subset of hyperinsulinemic patients, but this might not have clinical importance, since statistical significance was borderline and was observed only after adjustment for BMI.

Some previous studies indicate that proinsulin conversion intermediates may be associated with risk of CAD (12). The assay method used in this study does not separate proinsulin conversion intermediates from proinsulin. Rather, we report "proinsulin-like material," which comprises both intact proinsulin and the des-31,32-proinsulin split product. The des-64,65-proinsulin conversion intermediate was assessed using a different antiserum (18D), but it appears in negligible amounts and with immunoreactivity not found to be different among groups (data not shown).

The high prevalence of obesity and IGT was remarkable in both premature CAD (48%) and non-CAD (60%) groups. The obesity pattern was of the central (abdominal) type with a WHR of 0.88 in the CAD and 0.86 in the non-CAD groups. IGT was detected in both non-CAD and premature CAD groups (25 and 28%, respectively). This is significantly greater than the prevalence of IGT reported in the general population (11.2%) (30).

There are several limitations to this case-control study that may affect the data and its interpretation. As with most case-control studies, there may be undetected confounding variables and biases. The selection process for the control group without CAD may have introduced bias due to the strict inclusionary criteria requiring either a negative results on cardiac catheterization or a normal thallium exercise test. The advantage of this selection process was to exclude control subjects who might have unrecognized silent ischemic heart disease. The disadvantage, however, was that our normal subjects without CAD included patients from the clinic or hospital with suspicion of CAD who had either atypical chest pain, an abnormal rest or stress electrocardiogram (ECG), and ≥1 major CAD risk factor. This resulted in recruiting patients who were likely to be obese with hyperinsulinemia and its associated factors. To compensate for the selection process, we included for comparison results from an additional reference volunteer nonhospital nondiabetic population. Another limitation of the case-control approach is that while the design clearly distinguishes subjects with premature CAD from those without premature CAD, there is no longitudinal follow-up and, thus it remains unknown whether our hyperinsulinemic control subjects without CAD are likely to develop CAD in the future. It could be speculated, for example, that early-onset CAD is dependent on hyperlipidemia, a family history of CAD, and diabetes, whereas pancreatic β-cell secretory hormones potentially have increased importance for atherosclerosis later in life.

One other explanation for the hyperinsulinemia in the non-CAD group could be inclusion of normal subjects with chest pain and normal coronary arteries, the cardiological syndrome X. Recent studies have noted the high prevalence of hyperinsulinemia in this group with an overlap of several of the features of the endocrine syndrome X (31,32). Upon chart review, few, if any, of our patients met the criteria for the cardiological syndrome X (atypical chest pain, objective evidence of ischemia with stress testing on an ECG and/or thallium study, and normal coronary arteries at catheterization), making this an unlikely case for the observed hyperinsulinemia.

In summary, hyperinsulinemia and hyperproinsulinemia are common in both male and female nondiabetic patients with premature CAD. However, elevation of these hormones appears equally prevalent in patients with traditional CAD risk factors but no detectable coronary atherosclerosis. Increased INS values did not discriminate between the odds of being in either the premature CAD or the non-CAD group. Few β-cell secretory abnormalities were specifically associated with the risk of premature CAD. Only proinsulin was associated with the risk of premature CAD, but this was restricted to the postglucose measurements and was of borderline statistical significance. Both the CAD and non-CAD populations were obese, which may explain, at least in part, the high prevalence of insulin resistance and pancreatic β-cell secretory product elevations. It appears that hyperinsulinemia tends to cluster with the factors described in the insulin resistance syndrome, and obesity appears as a common link with the measured hormonal-metabolic abnormalities. From our case-control data, the hypothesis that insulin and proinsulin are independent promoters or markers of atherosclerosis remains questionable. While pancreatic β-cell secretory hormones may be necessary for development of premature CAD, none is sufficient for the production of atherosclerosis. Rather, the combination of hyperinsulinemia and hyperproinsulinemia along with other CAD risk factors and a genetic predisposition may be needed for the development of early-onset atherosclerosis.

ACKNOWLEDGMENTS

This study was supported by National Institutes of Health RO1 HL49765-01 and MO1 RR 08084. R.J.K., R.E.R., and R.M.C. are supported by National Institutes of Health Grant RO1 HL49765-01. R.M.C. is also supported by a Clinical Research grant from the American Diabetes Association and National Institutes of Health Grant M01 RR08084 (Children's Hospital-University of Cincinnati General Clinical Research Center).

REFERENCES

1. Pyorala K, Savolainen E, Kaukola S, Haapakoski J: Plasma insulin as a coronary heart disease risk factor: relationship to other risk factors and predictive value over 9.5 year follow up of the Helsinki Policemen Study population. *Acta Med Scand* 701 (Suppl.):7–14, 1985
2. Eschwege E, Richard JL, Thibult N: Coronary heart disease in relation with diabetes, blood glucose and plasma insulin levels: the Paris Prospective Study, ten years later. *Horm Metab Res* (Suppl.):41–46, 1985
3. Welborn TA, Wearne K: Coronary heart disease and cardiovascular mortality in Busselton with reference to glucose and insulin concentrations. *Diabetes Care* 2:154–160, 1979
4. Savage PJ, Saad MF: Insulin and atherosclerosis: villain, accomplice, or innocent bystander? *Br Heart J* 69:473–475, 1993
5. Jarrett RJ: Why is insulin not a risk factor for coronary heart disease? *Diabetologia* 37:945–947, 1994
6. Reaven GM, Laws A: Insulin resistance, compensatory hyperinsulinemia, and coronary heart disease. *Diabetologia* 37:948–952, 1994
7. Fontbonne A: Why can high insulin levels indicate a risk for coronary heart disease? *Diabetologia* 37:953–955, 1994
8. Stern MP: The insulin resistance syndrome: the controversy is dead, long live the controversy! *Diabetologia* 37:956–958, 1994
9. Zamboni M, Armellini F, Sheiban I, De Marchi M, Todesco T, Bergamo-Andreis IA, Cominacini L, Bosello O: Relation of body-fat distribution in men and degree of coronary artery narrowings in coronary artery disease. *Am J Cardiol* 70:1135–1138, 1992

10. Spallarossa P, Cordera R, Andraghetti G, Bertino G, Brunelli C, Caponnetto S: Association between plasma insulin and angiographically documented significant coronary artery disease. *Am J Cardiol* 74:177–179, 1994
11. Young MH, Jeng C-Y, Sheu WH, Shieh S-M, Fuh MM, Chen YI, Reaven GM: Insulin resistance, glucose intolerance, hyperinsulinemia and dyslipidemia in patients with angiographically demonstrated coronary artery disease. *Am J Cardiol* 72:458–460, 1993
12. Nagi DK, Hendra TJ, Ryle AJ, Cooper TM, Temple RC, Clark PMS, Schneider AE, Hales CN, Yudkin JS: The relationship of concentrations of insulin, intact proinsulin and 32–33 split proinsulin with cardiovascular risk factors in type 2 (non-insulin- dependent) diabetic subjects. *Diabetologia* 33:532–537, 1990
13. Haffner SM, Mykkanen L, Stern MP, Valdez RA, Heisserman JA, Bowsher RR: Relationship of proinsulin and insulin to cardiovascular risk factors in nondiabetic subjects. *Diabetes* 42:1297–1302, 1993
14. Ward WK, LaCava EC, Paquette TL, Beard JC, Wallum BJ, Porte D Jr: Disproportionate elevation of immunoreactive proinsulin in type 2 (non-insulin-dependent) diabetes mellitus and in experimental insulin resistance. *Diabetolgia* 30:698–702, 1987
15. Temple RC, Clark PMS, Nagi DK, Schneider AE, Yudkin JS, Hales CN: Radioimmunoassay may overestimate insulin in non-insulin-dependent diabetics. *Clin Endocrinol* 32:689–693, 1990
16. Haffner SM, Mykkanen L, Valdez RA, Stern MP, Holloway DL, Monterrosa A, Bowsher R: Disproportionately increased proinsulin levels are associated with the insulin resistance syndrome. *J Clin Endocrinol Metab* 79:1806–1810, 1994
17. Starr JI, Horowitz DL, Rubenstein AH, Mako ME: Insulin, proinsulin and C-peptide. In *Methods of Hormone Radioimmunoassay*. 2nd ed. Jaffe BM, Behrman HR, Eds. Orlando, FL, Academic, 1979, p. 613–642
18. Cohen RM, Given BD, Licinio-Paixao J, Provow SA, Rue PA, Frank BH, Root MA, Polonsky KS, Tager HS, Rubenstein AH: Proinsulin radioimmunoassay in the evaluation of insulinomas and familial hyperproinsulinemia. *Metabolism* 35:1137–1146, 1986
19. Bowsher RR, Wolny JD, Frank BH: A rapid and sensitive radioimmunoassay for the measurement of proinsulin in human serum. *Diabetes* 41:1084–1090, 1992
20. Faber OK, Binder C, Markussen J, Heding LG, Naithani VK, Kuzuya H, Blix P, Horwitz DL, Rubenstein AH: Characterization of seven C-peptide antisera. *Diabetes* 27:170–177, 1978
21. World Health Organization: *Diabetes Mellitus: Report of a WHO Study Group*. Geneva, World Health Org., 1985 (Tech. Rep. Ser., no. 727)
22. Stout RW, Valliance-Owen W: Insulin and atheroma. *Lancet* i:1078–1080, 1969
23. Ronnemaa T, Laasko M, Pyorala K, Kallio V, Puukka P: High fasting plasma insulin is an indicator of coronary artery disease in non-insulin-dependent diabetic patients and nondiabetic subjects. *Arterioscler Thromb* 11:80–90, 1991
24. Pyorala K: Relationship of glucose tolerance and plasma insulin to the incidence of coronary heart disease: results from two population studies in Finland. *Diabetes Care* 2:154–160, 1979
25. Van Cauter E, Mestrez F, Sturis J, Polonsky KS: Estimation of insulin secretion rates from C-peptide levels. *Diabetes* 41:368–377, 1992
26. Terris S, Steiner DF: Retention and degradation of ^{125}I-insulin by perfused livers from diabetic rats. *J Clin Invest* 57:885–896, 1976
27. Revers RR, Henry R, Schmeiser L, Kolterman O, Cohen R, Bergenstal R, Polonsky K, Jaspan J, Rubenstein A, Frank B, Galloway J, Olefsky JM: The effects of biosynthetic human proinsulin on carbohydrate metabolism. *Diabetes* 33:762–770, 1984
28. Bergenstal RM, Cohen RM, Lever E, Polonsky JB, Jaspan J, Blix P, Revers O, Kolterman J, Olefsky J, Steiner K, Cherrington A, Frank BH, Galloway J, Rubenstein AH: The metabolic effects of biosynthetic human proinsulin in individuals with type I diabetes. *J Clin Endocrinol Metab* 58:973–979, 1984
29. Polonsky KS, Licinio-Paixao J, Given BD, Pugh W, Rue P, Galloway J, Karrison T, Frank B: Use of biosynthetic C-peptide in the measurement of insulin secretory rates in normal volunteers and type I diabetic patients. *J Clin Invest* 77:98–105, 1986
30. Harris MI: Impaired glucose tolerance in the US population. *Diabetes Care* 12:464–474, 1989
31. Chauhan A, Foote J, Petch MC, Schofield PM: Hyperinsulinemia, coronary artery disease and syndrome X. *J Am Coll Cardiol* 23:364–368, 1994
32. Reaven GM: Syndrome X: is one enough? *Am Heart J* 127:1439–1442, 1994