Cross-Sectional and Prospective Associations Between Proinsulin and Cardiovascular Disease Risk Factors in a Population Experiencing Rapid Cultural Transition

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OBJECTIVE — To examine cross-sectional and prospective associations between proinsulin and cardiovascular disease risk factors using data from a population-based study of type 2 diabetes among Native Canadians.

RESEARCH DESIGN AND METHODS — Between 1993 and 1995, 72% of eligible members of a Native Canadian community participated in a baseline diabetes prevalence survey. Fasting samples were collected for glucose, C-peptide, proinsulin, lipids, and apolipoproteins. A 75-g oral glucose tolerance test was administered, and a second sample for glucose was drawn after 120 min. Blood pressure and waist circumference were determined. In the present study, subjects with normal glucose tolerance (NGT) ($n = 505$) and impaired glucose tolerance (IGT) ($n = 74$) were included in cross-sectional analyses. In 1998, 95 individuals who had IGT or NGT at baseline with an elevated 2-h glucose concentration (≥7.0 mmol/l) participated in a follow-up evaluation using the protocol used at baseline. Cross-sectional and prospective associations between proinsulin and cardiovascular risk factors were assessed using correlation and multiple linear regression analyses.

RESULTS — After adjustment for covariates including age, sex, C-peptide, waist circumference, and glucose tolerance status, fasting proinsulin concentration was significantly associated with concurrently measured lipid and apolipoprotein concentrations (triglycerides: $r = 0.18$, $P < 0.0001$; total cholesterol: $r = 0.10$, $P = 0.02$; LDL cholesterol: $r = 0.11$, $P = 0.01$; HDL cholesterol: $r = -0.16$, $P = 0.0002$; apolipoprotein (apo) B: $r = 0.17$, $P < 0.0001$; apoAI: $r = -0.11$, $P = 0.008$). In the adjusted prospective analysis, baseline triglycerides, HDL cholesterol, and apoB were associated with changes over time in proinsulin ($r = 0.23$, $P = 0.04$; $r = -0.30$, $P = 0.01$; $r = 0.23$, $P = 0.04$; respectively).

CONCLUSIONS — These results confirm previously reported cross-sectional associations between proinsulin and lipid concentrations. In addition, an unexpected association between baseline lipids and proinsulin change was documented.

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Ischemic heart disease and stroke account for half of the total mortality among individuals with type 2 diabetes in the U.S. (1). This highly unfavorable cardiovascular profile is foreshadowed by a period during which both diabetic and prediabetic individuals are dyslipidemic and hypertensive (2–4). It has been suggested that these abnormalities might be a consequence of the extended period of exposure to insulin resistance–related hyperinsulinemia (5,6). Although this hypothesis has been supported by the findings of both in vivo and in vitro studies (7–9), the issue remains controversial (10).

The assessment of the relationship between insulin and cardiovascular risk factors is complicated by the fact that conventional insulin radioimmunoassays display a high degree of cross-reactivity with proinsulin and its split-products (11). Considering that proinsulin concentrations are disproportionately elevated in subjects with diabetes (12–18) and (in some studies) impaired glucose tolerance (IGT) (13–15,17,18), it is conceivable that this prohormone may have particularly detrimental metabolic effects in the pathogenesis of cardiovascular disease (CVD). Support for this theory emerged during the 2-year interim analysis of a clinical trial comparing human proinsulin and human insulin (19). Six myocardial infarctions (including two deaths) occurred in the human proinsulin group, and none occurred in the comparison group.

Cross-sectional relationships between proinsulin and intermediate quantitative traits associated with CVD have

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Abbreviations: apo, apolipoprotein; BP, blood pressure; CVD, cardiovascular disease; FFA, free fatty acid; GTS, glucose tolerance status; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test.

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

been examined in a limited number of studies (20–24). Among both diabetic and nondiabetic subjects, proinsulin has exhibited moderate but significant associations with blood pressure (BP) and concentrations of total cholesterol, triglycerides, LDL and HDL cholesterol (20–24), and plasminogen activator inhibitor type 1 (25) independent of other factors included in multivariate analysis. However, the role of elevated proinsulin in atherogenesis remains controversial (23,26).

Two important research questions have yet to be addressed in this body of literature. First, are proinsulin concentrations associated with CVD risk factors among North American aboriginal people, a population with increasing rates of both type 2 diabetes (27) and coronary heart disease (28)? Second, what are the prospective relationships between proinsulin and CVD risk factors in nondiabetic subjects who are at risk of developing diabetes? We examined these specific issues using data from the cross-sectional and longitudinal components of the Sandy Lake Health and Diabetes Project (29). The residents of Sandy Lake are Native Canadians from the isolated subarctic region of northern Ontario, a population that has traditionally experienced relatively low rates of cardiovascular mortality (30). Westernization is occurring rapidly in this region, however, and has resulted in an epidemiological transition characterized by dramatic increases in the prevalence of obesity and type 2 diabetes (31,32). Although rates of dyslipidemia and hypertension are remarkably low among normoglycemic individuals in this population, the situation is quite different in the presence of glucose intolerance (33). Indeed, despite the relatively recent emergence of the diabetes epidemic in this area (31,32), it is becoming apparent that this disease is already having a detrimental effect on the cardiovascular health of its residents. Between 1984 and 1995, rates of ischemic heart disease in this population increased from 76/10,000 to 186/10,000 (34).

RESEARCH DESIGN AND METHODS

Baseline prevalence survey, 1993–1995

The methodology of the Sandy Lake Health and Diabetes Project prevalence study has been presented in detail in previous publications (29). Between July 1993 and December 1995, 728 of 1,018 residents (72%) of Sandy Lake aged 10–79 years participated in a population-based cross-sectional survey to determine the prevalence of type 2 diabetes and its associated risk factors. Signed informed consent was obtained from all participants, and the study was approved by the Sandy Lake First Nation Band Council and University of Toronto Ethics Review Committee. The cross-sectional component of the current study is based on data from the 579 individuals identified as having normal glucose tolerance (NGT) or IGT in this survey and for whom specimens were available for proinsulin determination.

Participants provided fasting blood samples for glucose, C-peptide, proinsulin, and lipids after an 8- to 12-h overnight fast. A 75-g oral glucose tolerance test (OGTT) was administered, and a second sample for glucose was drawn after 120 min. Individuals were excluded from the OGTT if they had physician-diagnosed diabetes and if *1*) they were currently receiving treatment with insulin or oral hypoglycemic agents or 2) they had a fasting blood glucose concentration exceeding 11.1 mmol/l. Women who were pregnant at the time of initial contact received their OGTT 3 months postpartum. Diabetes and IGT were diagnosed according to World Health Organization criteria (35).

C-peptide concentration was measured using a radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA), which has a minimal detection limit of 43 pmol/l and cross-reactivities of 0% with insulin and <13% with proinsulin. Proinsulin was determined using a human proinsulin radioimmunoassay, which has a laboratory sensitivity of 3.5 pmol/l and a coefficient of variation of 6.2–21.0% (Linco Research, St. Louis, MO). This assay displays 46% cross-reactivity with des 31,32 proinsulin, the major form of circulating split proinsulin, and thus, reported values refer to total proinsulin-like materials (36). Cross-reactivity of this assay with des 64,65 proinsulin, insulin, and C-peptide is very low (<0.1%). Proinsulin was measured in serum specimens that had been stored at −70°C for 3–5 years at the Core Lab of the Banting and Best Diabetes Center, University of Toronto. Glucose concentration was determined using the glucose oxidase method. Cholesterol, triglycerides, and HDL cholesterol concentrations were determined using methods described in the Lipid Research Clinics Manual of Operations (37). LDL cholesterol concentration was calculated using the Friedewald formula (38), and concentrations of apolipoprotein (apo)B and apoAI were determined using nephelometry (37).

Anthropometric measurements were performed with the participant wearing either undergarments and a hospital gown or light athletic clothing and no shoes. Each measurement was performed twice, and the average was used in the analysis. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Weight was measured to the nearest 0.1 kg using a hospital balance-beam scale. BMI was defined as weight/height^2 (kg/m^2). The waist was measured to the nearest 0.5 cm at the point of narrowing between the umbilicus and xiphoid process (as viewed from behind); the hips were measured to the nearest 0.5 cm at the maximum extension of the buttocks. Waist-to-hip ratio was calculated as the ratio of these two circumferences.

BP was measured in the right arm with the participant seated and the arm bared. Systolic BP was recorded to the nearest 2 mmHg at the appearance of the first Korotkoff sound (phase I), and diastolic BP was recorded to the nearest 2 mmHg at the appearance of the fifth Korotkoff sound (phase V). Two measurements were performed using a handheld aneroid sphygmomanometer, and the average of the two was used in the analysis.

Follow-up survey, 1998

During the summer of 1998, Sandy Lake residents who, at the time of the baseline survey, had IGT (n = 74) or NGT ≥7.0 mmol/l (n = 51) based on a 2-h postchallenge glucose concentration were invited to participate in a follow-up visit to determine current glucose tolerance status (GTS). The selection of these subjects for follow-up was based on an interest in the natural history of IGT in this population as well as a request by the Band Council to reexamine high-risk individuals. Of the 125 individuals in the follow-up cohort, 3 (3 IGT, 0 NGT) had died, 11 (9 IGT, 2 NGT) were no longer living in the community, 2 (1 IGT, 1 NGT) were too sick to participate, and 14 (6 IGT, 8 NGT) re-

Table 1—*Metabolic and cardiovascular characteristics of subjects with normal and impaired glucose tolerance from the cross-sectional phase of the Sandy Lake Health and Diabetes Project*

	NGT (n = 505; 232 men and 273 women)		IGT (n = 74; 16 men and 58 women)		
Variable*	Mean	SD	Mean	SD	*P*
Age at screening (years)	25.59	12.92	39.10	17.68	<0.0001
Proinsulin (pmol/l)	12.04	7.15	19.00	12.37	<0.0001
C-peptide (pmol/l)	599.48	372.52	841.21	442.79	<0.0001
Cholesterol (mmol/l)	4.29	0.88	4.77	0.71	<0.0001
Triglyceride (mmol/l)	1.26	0.61	1.68	0.78	<0.0001
LDL cholesterol (mmol/l)	2.48	0.75	2.78	0.59	0.01
HDL cholesterol (mmol/l)	1.26	0.27	1.23	0.29	0.31
ApoB (g/l)	1.00	0.27	1.18	0.24	<0.0001
ApoA1 (g/l)	1.47	0.21	1.53	0.27	0.10
Waist circumference (cm)	88.56	13.60	97.62	10.76	<0.0001
Systolic BP (mmHg)	113.72	13.71	124.25	17.55	<0.0001
Diastolic BP (mmHg)	64.50	11.32	69.47	12.67	0.0006

Data are *n*. *All metabolic variables are fasting concentrations.

fused to attend. Therefore, 95 members (76%) of this high-risk cohort participated in the follow-up examination. Nonparticipants did not differ significantly from participants in age, sex, or anthropometric or metabolic variables (data not shown). Concentrations of metabolic and cardiovascular variables and anthropometric measurements were assessed using field and laboratory methods and equipment identical to those used during the baseline survey. Signed informed consent was obtained from all subjects, and the follow-up protocol was approved by the Sandy Lake First Nation Band Council and the University of Toronto Ethics Review Committee.

Statistical analyses

The analytical convention in this body of literature is to "adjust" proinsulin concentration for insulin secretion, usually by employing the proinsulin-to-insulin ratio. This is problematic for two reasons. First, peripheral insulin concentrations do not adequately represent insulin secretion, given that "insulin undergoes a large and variable hepatic extraction as well as peripheral clearance that varies under different physiological circumstances" (39). Second, Kronmal has pointed out that the use of ratio variables in correlation and regression can result in spurious findings (40). Therefore, we opted to avoid the use of ratios and to analyze the effect of proinsulin on concentrations of CVD risk factors after adjustment for C-peptide, which is "co-secreted with insulin in an equimolar ratio, is not extracted by the liver, and has a constant peripheral clearance" (39).

The association between proinsulin concentrations and CVD risk factors was investigated using concurrent measures collected during the 1993–1995 baseline prevalence survey, described below as the "cross-sectional study." The prospective associations of baseline concentrations and changes over 4 years in proinsulin and CVD risk factors were investigated using data from both the baseline prevalence (1993–1995) and follow-up (1998) surveys; these analyses are described below as the "prospective study." All analyses were performed using SAS version 6.12 software (SAS Institute, Cary, NC) (41).

Cross-sectional study

The distributions of continuous variables were assessed for normality, and the natural log transformations of skewed variables were used in subsequent analyses. Crude and adjusted associations between concurrent measures of proinsulin and cardiovascular risk factors (lipids, apo, and BP) were assessed using correlation coefficients and multiple linear regression. For each dependent variable, three models were constructed that included the following independent variables: *1*) proinsulin alone; 2) proinsulin, age, sex, and C-peptide, and *3*) proinsulin, age, sex, C-peptide, waist circumference, and GTS. Waist circumference was used in lieu of waist-to-hip ratio as a measure of abdominal adiposity, given that the former is superior as an estimate of intra-abdominal fat mass (42). We tested for statistical interactions between *1*) sex and proinsulin and *2*) GTS and proinsulin by adding interaction terms to each of the final models, as well as by examining stratum-specific results. Because none of the interaction parameter estimates were significant at the 5% level (all $P > 0.51$) and the results of stratum-specific models were not markedly different, we present models with the GTS categories and sexes pooled. Model collinearity was assessed using the variance inflation factor method described by Freund and Littell (43). Based on these criteria, none of models displayed evidence of marked collinearity.

Prospective study

Changes in metabolic and cardiovascular variables were calculated as the follow-up level (1998) minus the baseline level (1993–1995). The associations were assessed between *1*) change in proinsulin concentration and cardiovascular risk variables during the follow-up period; *2*) baseline proinsulin concentration and changes in cardiovascular risk variables over time; and *3*) baseline levels of cardiovascular risk variables and change in proinsulin concentration over time. Due to skewness in the distribution of both predictor and outcome variables, Spearman correlation coefficients were used to assess these relationships, with and without adjustment for age, sex, C-peptide concentration, waist circumference, baseline and follow-up diabetes status, and baseline levels of dependent and independent change variables. Duration of follow-up (median 4.2 years) did not have any effect on these associations (data not shown).

RESULTS

Cross-sectional study

Characteristics of subjects included in the cross-sectional study are presented in Table 1. Individuals with IGT were older and had higher waist circumference values and both systolic and diastolic BP compared with subjects with NGT. In addition, subjects with IGT had higher concentrations of proinsulin, C-peptide,

Table 2—*Crude and adjusted cross-sectional relationships between fasting proinsulin concentration and cardiovascular risk factors in nondiabetic subjects (n = 577), Sandy Lake Health and Diabetes Project*

Dependent variable*	Adjustment†	Statistics for proinsulin variable*				Full-model R^2
		β	*t*	Partial *r*	*P*	
Cholesterol		0.0729	4.51	0.19	<0.0001	0.03
	Age, sex, C-peptide	0.0561	3.25	0.14	0.0012	0.32
	Age, sex, C-peptide, waist, GTS	0.0445	2.42	0.10	0.0157	0.33
Triglycerides		0.3777	11.13	0.42	<0.0001	0.18
	Age, sex, C-peptide	0.2274	6.04	0.25	<0.0001	0.31
	Age, sex, C-peptide, waist, GTS	0.1769	4.34	0.18	<0.0001	0.32
LDL cholesterol		0.1090	4.61	0.20	<0.0001	0.03
	Age, sex, C-peptide	0.0958	3.70	0.15	0.0002	0.29
	Age, sex, C-peptide, waist, GTS	0.0688	2.53	0.11	0.0116	0.32
HDL cholesterol		−0.1406	−8.33	−0.33	<0.0001	0.11
	Age, sex, C-peptide	−0.1122	−5.28	−0.22	<0.0001	0.13
	Age, sex, C-peptide, waist, GTS	−0.0830	−3.78	−0.16	0.0002	0.18
ApoB		0.1695	8.17	0.32	<0.0001	0.10
	Age, sex, C-peptide	0.1250	5.68	0.23	<0.0001	0.38
	Age, sex, C-peptide, waist, GTS	0.0928	4.06	0.17	<0.0001	0.41
ApoA1		−0.0527	−4.31	−0.17	<0.0001	0.03
	Age, sex, C-peptide	−0.0487	−3.24	−0.13	0.0013	0.10
	Age, sex, C-peptide, waist, GTS	−0.0424	−2.66	−0.11	0.0080	0.11
Systolic BP		0.0409	4.10	0.16	<0.0001	0.03
	Age, sex, C-peptide	0.0389	3.61	0.15	0.0003	0.30
	Age, sex, C-peptide, waist, GTS	0.0141	1.27	0.05	0.2033	0.35
Diastolic BP		0.0470	3.26	0.14	0.0009	0.02
	Age, sex, C-peptide	0.0223	1.32	0.06	0.1880	0.17
	Age, sex, C-peptide, waist, GTS	−0.0020	−0.12	−0.01	0.9082	0.20

*Natural log transformations used in analyses correct skewness; †natural log transformations of age and C-peptide used in analysis correct skewness.

lipids, and apolipoproteins (except HDL cholesterol) compared with those with NGT.

Results of correlation and multiple linear regression analyses examining crude and adjusted cross-sectional relationships between fasting proinsulin concentration and CVD risk factor levels in nondiabetic subjects are shown in Table 2. In unadjusted analyses, proinsulin was significantly and positively correlated with triglycerides, total and LDL cholesterol, and apoB and was significantly negatively correlated with HDL cholesterol and apoAI. These relationships were attenuated slightly but remained significant after controlling for age, sex, C-peptide concentration, waist circumference, and GTS (triglycerides: partial $r = 0.18$, $P < 0.0001$; total cholesterol: partial $r = 0.10$, $P = 0.0157$; LDL cholesterol: partial $r = 0.11$, $P = 0.0116$; HDL cholesterol: partial $r = -0.16$, $P = 0.0002$; apoB: partial $r = 0.17$, $P < 0.0001$; apoAI, $r = -0.11$, $P = 0.008$). There was no evidence of a threshold effect for any of these variables across quartiles of proinsulin (data not shown). In the fully adjusted model, proinsulin concentration explained ~3% (partial R^2) of the variation in concentrations of HDL cholesterol, triglycerides, and apoB. Proinsulin concentrations accounted for smaller amounts of independent variation in total cholesterol, LDL cholesterol, and apoAI (1–2%). Proinsulin displayed a positive, unadjusted association with systolic and diastolic BP, although associations were substantially attenuated after adjustment for waist circumference and other covariates.

Prospective study

The characteristics of participants in the follow-up survey, including mean levels of metabolic and cardiovascular variables measured at baseline, as well as mean changes in these variables over the 4-year follow-up period are shown in Table 3. The median follow-up time was 4.2 years, and diabetes developed in 24 of 95 subjects during this period. In addition, Table 3 (columns A–C) shows associations between baseline levels and changes over time in these variables after adjustment for covariates including age, sex, changes in C-peptide concentration and waist circumference, baseline and follow-up diabetes status, and baseline level of dependent and independent change variables. Although change in proinsulin was significantly associated with changes in concentrations of total cholesterol (partial $r = 0.31$, $P < 0.01$), triglycerides (partial $r = 0.25$, $P < 0.05$), LDL cholesterol (partial $r = 0.24$, $P = 0.04$), and apoB (partial $r = 0.26$, $P = 0.02$) (Table 3, column *A*), baseline proinsulin concentration was not significantly associated with changes in cardiovascular risk factors (Table 3, column *B*). However, after adjustment for covariates, baseline concentrations of triglycerides, HDL cholesterol, and apoB were significantly associated with changes over time in proinsulin concentration (partial $r = 0.23$, $P = 0.04$; partial $r = -0.30$, $P < 0.01$; partial $r = 0.23$, $P = 0.04$, respectively) (Table 3, column *C*).

CONCLUSIONS— In the present study, we have reported significant cross-

Table 3—*Metabolic characteristics and cardiovascular risk factors of subjects with normal and impaired glucose tolerance from the follow-up phase of the Sandy Lake Health and Diabetes Project (29 men; 66 women) and associations between baseline levels and changes over time in these variables

	Baseline values		Change over 4-year follow-up period		A†: Correlation between change in proinsulin and change in CVD variable		B‡: Correlation between baseline proinsulin and change in CVD variable		C†: Correlation between baseline CVD variable and change in proinsulin	
Variable	Mean	SD	Mean	SD	Partial *r*	*P*	Partial *r*	*P*	Partial *r*	*P*
NGT/IGT/diabetes (*n*)	41/54/0		54/17/24							
Age at baseline (years)	35.10	16.97	—	—						
Waist (cm)	96.35	11.37	3.16	5.78						
C-peptide (pmol/l)	831.72	438.46	299.25	485.26						
Proinsulin (pmol/l)	17.06	11.60	3.66	18.40						
Cholesterol (mmol/l)	4.70	0.85	0.09	1.38	0.31	<0.01	−0.11	0.33	0.10	0.39
Triglycerides (mmol/l)	1.61	0.75	0.29	2.20	0.25	0.02	0.03	0.79	0.23	0.04
LDL cholesterol (mmol/l)	2.78	0.68	−0.08	0.54	0.24	0.04	−0.12	0.31	0.18	0.10
HDL cholesterol (mmol/l)	1.19	0.26	0.02	0.20	0.11	0.32	−0.03	0.81	−0.30	<0.01
ApoB (g/l)	1.16	0.27	−0.05	0.19	0.26	0.02	−0.19	0.09	−0.23	0.04
ApoAI (g/l)	1.49	0.22	−0.02	0.17	0.20	0.08	−0.13	0.25	−0.19	0.09
Systolic BP (mmHg)	121.00	15.48	4.68	13.10	0.13	0.23	−0.09	0.43	−0.14	0.23
Diastolic BP (mmHg)	68.74	11.08	4.74	11.51	0.11	0.34	0.07	0.52	−0.02	0.83

*Spearman correlation analysis, sample sizes vary slightly due to occasional missing values; †analyses adjusted for age, sex, change in C-peptide concentration, change in waist circumference, baseline and follow-up diabetes status, and baseline level of dependent and independent change variables; ‡analyses adjusted for age sex, C-peptide concentration, change in waist circumference, baseline and follow-up diabetes status, and baseline level of dependent and independent change variables.

sectional associations of fasting proinsulin with lipid and apo concentrations in nondiabetic subjects after adjustment for covariates including age, sex, insulin secretion, and intra-abdominal obesity. To our knowledge, these relationships have not previously been described in North American indigenous people, a population undergoing rapid epidemiological transition, nor have associations between proinsulin and apo been reported. Furthermore, for the first time, we have documented prospective relationships between change in proinsulin concentration and change in CVD risk factors, as well as associations between baseline concentrations of triglycerides, HDL cholesterol, and apoB, and change in proinsulin over time. Our findings are consistent with the results of previous research examining cross-sectional relationships between proinsulin concentration and cardiovascular risk factors in nondiabetic subjects. In the three studies that presented results adjusted for age, sex, and adiposity (21–23), proinsulin concentration was consistently related to triglyceride concentration, and independent associations were reported between proinsulin and LDL and HDL cholesterol in two of the three papers (22,23). We did not find significant cross-sectional or prospective associations between proinsulin and either systolic or diastolic BP, a result that may be related to the low prevalence of hypertension in this population (33).

To date, the physiological mechanism behind the consistent and significant association between proinsulin and lipids is unknown. It is possible that a common factor is responsible for both increased proinsulin and increased lipid concentrations. We have previously described positive relationships between waist circumference and both lipid (33) and proinsulin concentrations (unpublished observations) in this population. Therefore, it is conceivable that intra-abdominal fat is playing such a role. Haffner et al. (44) have proposed such a mechanism for intra-abdominal fat in explaining increased levels of proinsulin, plasminogen activator inhibitor type 1, and intimal-medial wall thickness. In our study, however, the associations between proinsulin and CVD risk factors remained significant after adjustment for waist circumference (a reliable indicator of intra-abdominal fat) in both cross-sectional and prospective analyses. This finding suggests that the association between proinsulin and CVD risk factors might be at least partially independent of intra-abdominal fat.

It is also possible that elevated proinsulin concentration may be directly involved in the development of dyslipidemia, atherosclerosis, thrombosis, or related metabolic disorders. Despite the relatively low concentrations of this prohormone in nondiabetic subjects and its limited metabolic activity (45), the metabolic clearance rate of proinsulin is substantially lower than that of insulin, possibly resulting in prolonged exposure of susceptible tissues to proinsulin molecules (45). This hypothesis is further supported by in vitro data, suggesting that insulin propeptides stimulate the "synthesis and secretion of fibrinolytic proteins by hepatocytes and endothelial cells" (45–47), and in vivo findings from both diabetic and nondiabetic subjects, indicating significant associations between proinsulin and plasminogen activator inhibitor type 1 (25,48). In recent research, significant associations between proinsulin concentration and LDL particle size have been documented (49), and it has been suggested that this relation-

ship may operate through the hypertriglyceridemia that characterizes states of β-cell dysfunction (49). Triglycerides are an important determinant of LDL particle size (49), and previous research demonstrated that impaired insulin secretion was associated with higher nonesterified fatty acid concentrations in men, which, in turn, was related to increased triglyceride concentrations (50). Confirmation of the hypothesis of an independent predictive role of proinsulin in the development of dyslipidemia will require the demonstration of a prospective association between baseline proinsulin concentration and change in lipid concentrations over time after adjustment for confounding factors. We were unable to demonstrate such an association in the small prospective study reported here. Instead, we found that change in proinsulin concentration during the follow-up period was significantly associated with changes in lipid concentrations, indicating that proinsulin and lipid concentrations seem to increase concurrently.

Finally, it is conceivable that the pathogenic mechanism may operate in the opposite direction, with higher baseline circulating lipid and apo concentrations leading to subsequent β-cell dysfunction and elevations in proinsulin concentrations. In the ZDF rat, short-term exposure to high levels of circulating free fatty acids (FFAs) causes β-cell hyperplasia and hyperinsulinemia, but chronic exposure and subsequent increases in FFA levels lead to functional and morphologic changes in β-cells and consequent diabetes (51). Substantial fat deposition in islets of obese ZDF rats has been documented, as well as the demonstration of FFA-induced loss of glucose-stimulated insulin secretion in these animals (52). Increased FFA levels also induce nitric oxide synthase, and Shimabukuro et al. (53,54) have shown that increased FFA levels in rat β-cells cause increases in both nitric oxide levels and ceramide-mediated β-cell apoptosis (programmed cell death). The consequent reduction in the number of β-cells may result in elevated proinsulin concentration, in that the rate of secretion by remaining cells is increased, thereby decreasing the intracellular stores and forcing the release of incompletely processed materials (55). Our findings of significant independent associations of baseline concentrations of triglycerides, LDL cholesterol, and apoB with changes in proinsulin concentration over time are consistent with this pathophysiological mechanism.

In summary, this study has demonstrated significant independent cross-sectional relationships between fasting proinsulin concentrations and CVD risk factors. In addition, we found that baseline concentrations of triglycerides, HDL cholesterol, and apoB were significantly associated with changes in proinsulin concentration over time. These data raise the possibility that lipotoxicity to the β-cell may help explain previous cross-sectional associations between lipid and proinsulin concentrations. However, the associations were modest and were demonstrated in the most glucose-intolerant segment of the nondiabetic population and, therefore, will require confirmation in future studies.

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