Association of Insulin and Insulin Propeptides With an Atherogenic Lipoprotein Phenotype

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A characteristic lipoprotein phenotype, including hypertriglyceridemia, a low high-density lipoprotein (HDL) cholesterol concentration, and a predominance of small, dense low-density lipoprotein (LDL) particles, is linked to insulin resistance and hyperinsulinemia. Individuals with these characteristics are supposed to be at increased risk of developing coronary heart disease (CHD). To address this issue further, relations between basal and postload glucose, insulin and insulin propeptide concentrations, and subfractions of apolipoprotein (apo) B–containing lipoproteins were examined in 62 consecutive Swedish nondiabetic men who had experienced a first myocardial infarction before the age of 45. A total of 41 age-matched, population-based healthy men were investigated as controls. Highly specific immunoradiometric assays were used for measuring intact proinsulin and des 31,32proinsulin levels. In all, 39% of the patients were found to be glucose-intolerant, and basal and postload hyper(pro)insulinemia were characteristic features irrespective of glucose tolerance category. Hypertriglyceridemic (HTG) lipoprotein phenotypes with a low HDL cholesterol concentration dominated among the patients, and hyperinsulinemia was linked to hypertriglyceridemia and putatively atherogenic lipoprotein traits, such as increased particle numbers of small very–low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL) and triglyceride enrichment of LDL. The corollary of these findings is that insulin resistance is a characteristic feature of young postinfarction patients and is accompanied by a complex atherogenic lipoprotein phenotype, new components of which are an abundance of small cholesteryl ester–rich VLDL and an elevated LDL triglyceride concentration.

LARGE-SCALE PROSPECTIVE epidemiological studies indicate that hyperinsulinemia[1,2] and glucose intolerance[3] are predictors of coronary heart disease (CHD) in middle-aged men. Furthermore, several studies have demonstrated an association between hyperinsulinemia (either fasting or after an oral glucose load) and established risk factors for cardiovascular disease, including hypertriglyceridemia, abdominal obesity, hypertension, and a reduced concentration of high-density lipoprotein (HDL) cholesterol.[4-6] Recently, it has also been demonstrated that plasma concentrations of insulin propeptides relate more closely to various cardiovascular risk factors in type II diabetics[7] and nondiabetics[8] than does insulin.

Hypertriglyceridemia and reduced plasma HDL cholesterol concentrations have been shown to be associated with insulin resistance and/or hyperinsulinemia in individuals with and without premature coronary artery disease.[9,10] A predominance of small, dense low-density lipoprotein (LDL) particles, which is frequently found in the plasma of CHD patients,[11-13] is also associated with insulin resistance and hyperinsulinemia[14] and probably constitutes a link between hyperinsulinemia and atherosclerosis.[15,16] Austin et al[17] have proposed that a gene locus that influences LDL subclass distribution likewise contributes to other lipoprotein and apolipoprotein abnormalities such as increased plasma apolipoprotein (apo) B and intermediate-density lipoprotein (IDL) levels and reduced plasma apo A-I or HDL cholesterol concentrations.[17] Moreover, the response of triglyceride-rich lipoproteins to fat intake together with the postheparin lipoprotein lipase activity are major determinants of LDL heterogeneity.[18]

Recent studies from our own unit have shown that the composition of small, cholesteryl ester–rich, very–low-density lipoprotein (VLDL) particles and dense LDL triglycerides is related to the severity of angiographically defined coronary artery disease, particularly among hypertriglyceridemic (HTG) patients.[19] Furthermore, our own data indicate that plasma concentrations of intact proinsulin and dense LDL triglycerides are independently related to the severity of coronary atherosclerosis in men with a previous myocardial infarction.[20] The purpose of the present study was to examine the hypothesis that glucose intolerance and hyperinsulinemia are implicated in determining an atherogenic lipoprotein phenotype in young men who suffer myocardial infarction. Associations were therefore sought between insulin, insulin propeptides, and subfractions of apo B–containing lipoproteins. Emphasis was placed on small VLDL, IDL, and dense LDL subfractions, which have been linked to disease severity in previous angiographic studies[21,22] and in this particular patient cohort.[19]

SUBJECTS AND METHODS

Study Population

Sixty-two men with a first myocardial infarction before the age of 45 were studied consecutively. Details of recruitment, representativity, and basic clinical characteristics have been described elsewhere.[19] Ninety-six healthy men with a similar age distribution (mean ± SD, 39.6 ± 2.7 years) were recruited by random selection

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of individuals born between 1947 and 1956 in Stockholm County (response rate, 69%). All participants completed a program that included medical history, clinical examination, blood sampling for analyses of major plasma lipoproteins, and an oral glucose tolerance test (OGTT), during which samples were drawn for determinations of insulin and insulin propeptides. The metabolic studies were performed 4 to 6 months after the acute event, when it was expected that the acute-phase reaction had subsided. Subfractions of plasma apo B–containing lipoproteins were only analyzed in the patient group, whereas insulin and insulin propeptide determinations were performed on all patients and the first 41 consecutively recruited control subjects.

Smoking habits were similar in the two groups at the time of metabolic investigations (31% and 33% current smokers among patients and controls, respectively). The cardioselective β-blocker metoprolol (50 to 100 mg daily) and acetyl salicylic acid (75 mg daily) were taken by all patients but three. Other anticoagulants or lipid-lowering drugs were not given.

Protocol

Blood samples for lipoprotein analyses were taken between 8 and 9 AM after 12 hours of fasting, during which time smokers were asked to refrain from smoking. All subjects had to be free from symptoms of infectious disease at the time of blood sampling. Venous blood for lipoprotein fractionation was drawn into precooled vacutainer tubes containing Na_2EDTA (1.4 mg/mL) and placed in an ice bath. Plasma was then recovered by low-speed centrifugation (1,400 × *g* for 20 minutes) at +1°C and kept at this temperature throughout the preparation procedures.

On a separate visit, glucose was ingested in a dose of 1.75 g/kg body weight in 150 to 200 mL water flavored with lemon extract, after 12 hours of fasting. Venous blood samples for determination of blood glucose, insulin, and insulin propeptides were obtained before and 15, 30, 45, 60, 90, and 120 minutes after glucose intake via an indwelling cannula inserted into an antecubital vein, with subjects remaining semirecumbent throughout the test. Blood was collected in vacutainer tubes containing heparin (143 USP units) for determination of blood glucose. Plasma samples for analyses of insulin and insulin propeptides were then prepared by low-speed ultracentrifugation (1,400 × *g* for 15 minutes) within 30 minutes and kept at −70°C until they were analyzed.

Biochemical Methods

The major plasma lipoproteins (VLDL, LDL, and HDL) were determined by a combination of preparative ultracentrifugation and precipitation of apo B–containing lipoproteins, followed by lipid analyses as previously described.[23] VLDL subfractions (VLDL Sf > 100, VLDL Sf 60-100, and VLDL Sf 20-60) and an IDL fraction were separated by density-gradient ultracentrifugation.[19] LDL subfractions were isolated using a modification[11] of the density-gradient ultracentrifugation procedure described by Chapman et al.[24] In short, LDL (d 1.019 to 1.063 kg/L) was first isolated from plasma. Fifteen LDL subfractions were then recovered with a fraction collector (FRAC-200; Pharmacia, Uppsala, Sweden) after ultracentrifugation of the gradients. Two major fractions were presented. Light LDL included LDL subfractions 1 to 9 (d 1.019 to 1.040 kg/L), and dense LDL contained subfractions 10 to 15 (1.040 to 1.063 kg/L). Lipid analyses of VLDL and LDL subfractions were made by enzymatic methods (14106-14108, Merck Diagnostica, Darmstadt, Germany; 877557, Boehringer Mannheim Diagnostica, Mannheim, Germany; and phospholipids 9990-54008, Wako Chemicals, Neuss, Germany). Total and soluble protein[25] levels were measured according to the method reported by Lowry et al.[26] The content of apo B was calculated as the difference between total and soluble protein.

Blood glucose level was measured by a glucose oxidase method (Kodak Ektachem; Eastman Kodak, Rochester, NY). Total immunoreactive insulin and C-peptide were determined by radioimmunoassays with polyclonal antisera supplied by Guildhay (Guildford, UK). Immunoradiometric assays were used to measure levels of intact proinsulin and des 31,32proinsulin.[27] Murine monoclonal antibodies A6 and 3B1 were obtained from Serono Diagnostics (Woking, UK), and murine monoclonal antibody PEP-001 was obtained from Novo Nordisk (Bagsvaerd, Denmark). The concentration of des 31,32proinsulin was calculated by subtracting the cross-reactivity of proinsulin (89%) in the des 31,32proinsulin assay. Des 31,32proinsulin cross-reacted (0.5%) in the intact proinsulin assay. A value for true insulin was calculated from the insulin measurements obtained by radioimmunoassay by subtraction of intact proinsulin and corrected des 31,32proinsulin. Specificity, precision, and reliability of the analytical methods have been described in detail.[28]

Cutoff limits for lipoprotein phenotyping were set to the 90th percentiles of the VLDL triglyceride (1.90 mmol/L) and LDL cholesterol (4.75 mmol/L) values in the control population (n = 96). The area under the curve (AUC) for the OGTT concentration profile was calculated for glucose, insulin, and insulin propeptides. Oral glucose tolerance was categorized as normal or decreased among the patients using the 90th percentile for the glucose AUC in the 96 population-based control men.

Computations and Statistical Analysis

Conventional methods were used for calculation of medians, means, and standard deviations. The composition of VLDL particles was calculated with the model developed by Miller and Small.[29] Insulin resistance was calculated from fasting plasma glucose and insulin concentrations using the Homeostasis Model Assessment.[30] Differences in continuous variables between groups were tested by Student's unpaired two-tailed *t* test, the nonparametric Mann-Whitney *U* test, or ANOVA with the Scheffe F test used as a post-hoc test. Analyses of covariance using body mass index (BMI) as the covariate were performed to control for the confounding effect of BMI on the case control and within-patient group differences obtained for the metabolic variables. Spearman rank correlation coefficients were calculated between glucose, insulin, and lipoprotein variables. The relations of glucose, insulin, and insulin propeptides to the lipoprotein variables were also estimated by calculating partial correlation coefficients with age and BMI as forced variables. Variables with a skewed distribution were always log-transformed before parametric statistical tests were conducted.

RESULTS

Patient and Control Characteristics

Plasma lipoprotein lipid concentrations and basal and postload levels of glucose, insulin, and insulin propeptides in patients and controls are shown in Table 1. A large proportion of the patients had hypertriglyceridemia and decreased oral glucose tolerance. For this reason, patients were divided according to the presence of normotriglyceridemic (NTG) and HTG lipoprotein phenotypes and normal and decreased oral glucose tolerance, respectively. By definition, HTG subjects had increased levels of VLDL lipids but also exhibited mildly elevated LDL cholesterol and low HDL cholesterol levels as compared with controls. NTG subjects had increased levels of LDL and slightly

Table 1. Plasma Lipoproteins, Basal Values and AUCs for OGTT Concentration Profiles for Insulin and Insulin Propeptides, and Insulin Resistance in Controls and in Patients Grouped According to Oral Glucose Tolerance or Lipoprotein Phenotype

Characteristic	Controls (n = 41)	Patients				P†
		Glucose Tolerance		Lipoprotein Phenotype		
		Normal (n = 38)	Decreased (n = 24)	NTG (n = 32)	HTG (n = 30)	
Age (yr)	39.4 ± 2.4	39.0 ± 4.1	39.8 ± 4.4	39.0 ± 4.6	39.6 ± 3.7	NS
Cholesterol (mmol/L)						
VLDL	0.37 (0.1-2.22)	0.71 (0.11-6.17)‡	0.80 (0.28-2.19)‡	0.48 (0.11-1.01)	1.50 (0.67-6.17)‡ **	<.001
LDL	3.63 ± 0.89	4.11 ± 1.10	4.58 ± 0.99§	4.31 ± 1.15¶	4.28 ± 1.0¶	<.001
HDL	1.12 ± 0.27	0.93 ± 0.24§	0.89 ± 0.22§	1.0 ± 0.26¶	0.84 ± 0.17‡ ‡‡	<.001
Triglycerides (mmol/L)						
VLDL	0.81 (0.26-2.22)	1.60 (0.27-13.5)‡	1.78 (0.58-4.54)‡	1.0 (0.27-1.89)	3.50 (1.98-13.5)‡ **	<.001
LDL	0.34 ± 0.13	0.44 ± 0.17¶	0.47 ± 0.12§	0.38 ± 0.12	0.53 ± 0.14‡ **	<.001
HDL	0.11 ± 0.04	0.13 ± 0.04	0.13 ± 0.03	0.12 ± 0.02	0.15 ± 0.04‡ ‡‡	<.05
Glucose (mmol/L)						
Basal	4.6 ± 0.4	4.8 ± 0.6	5.6 ± 0.7‡ ‖	5.0 ± 0.8¶	5.1 ± 0.7§	<.001
AUC	7,905 (264)	7,509 (270)	11,954 (321)‡ ‖	8,838 (412)	9,155 (537)¶	<.01
Insulin (pmol/L)						
Basal	33 (7.1)	63 (8.0)§	72 (11.5)‡	49 (8.8)	91 (8.4)‡ ††	<.001
AUC	32,554 (5,086)	54,997 (5,298)‡	64,397 (9,233)‡	48,538 (6,519)¶	71,291 (6,709)‡ ‡‡	<.001
Proinsulin (pmol/L)						
Basal	2.9 (0.9)	5.3 (0.6)‡	8.0 (1.2)‡	5.2 (1.1)§	7.6 (0.6)‡	<.001
AUC	1,506 (285)	2,589 (188)‡	3,267 (379)‡	2,460 (321)§	3,292 (190)‡	<.001
Des 31,32proinsulin (pmol/L)						
Basal	3.3 (1.2)	6.3 (1.0)§	6.5 (1.9)¶	4.8 (1.4)	8.7 (1.2)‡ ‡‡	<.001
AUC	2,371 (432)	4,073 (353)‡	4,524 (529)‡	3,588 (470)§	5,004 (324)‡ ‡‡	<.01
% insulin propeptides*						
Basal	18.1 (1.7)	17.6 (1.4)	20.1 (2.5)	17.2 (1.8)	15.6 (1.8)	NS
AUC	11.3 (0.7)	11.5 (0.7)	11.7 (1.1)	11.1 (0.9)	10.5 (0.8)	NS
Insulin resistance	6.8 (2.1-54.3)	13.2 (1.4-43.1)¶	17.8 (1.7-68.3)‡	10.8 (1.4-68.3)	20.6 (1.7-48.0)‡ ††	<.001
BMI (kg/m²)	24.5 ± 2.9	28.0 ± 4.1‡	28.6 ± 4.2‡	27.6 ± 4.4§	28.9 ± 3.7‡	<.001

NOTE. Values are the mean ± SD, geometric mean ± SEM, or range.

Abbreviation: NS, not significant.

*Intact proinsulin plus des 31,32proinsulin/total immunoreactive insulin.

†Unpaired two-tailed *t* test or the Mann-Whitney *U* test performed to identify differences between all patients and controls. Analysis of variance used to compare patients grouped according to glucose tolerance category or lipoprotein phenotype versus controls.

‡$P < .001$ *v* controls.

§$P < .01$ *v* controls.

¶$P < .05$ *v* controls.

‖$P < .001$ *v* patients with normal glucose tolerance.

**$P < .001$ *v* NTG patients.

††$P < .01$ *v* NTG patients.

‡‡$P < .05$ *v* NTG patients.

decreased HDL cholesterol levels as compared with controls. Of note, plasma lipoprotein lipid concentrations did not differ significantly between patients grouped according to oral glucose tolerance. Only 20 patients (32%) were classified as normolipidemic, as compared with 34 controls (82%). The corresponding figures for type IIa, IIb, and IV hyperlipidemias were 19%, 13%, and 36% among patients and 3%, 5%, and 10%, respectively, among controls.

Basal and postload hyperinsulinemia was found in the patients irrespective of oral glucose tolerance category, and was particularly prominent among HTG patients. The same pattern was found for basal and postload insulin propeptide concentrations. However, there was not a disproportionate increase in insulin propeptides over insulin in patients as compared with controls, either in the fasting state or when the entire AUC during the OGTT was considered. Insulin sensitivity was considerably lower in the patient group, particularly among HTG patients.

BMI was significantly higher in patients as compared with controls, but did not differ between patients grouped according to oral glucose tolerance or lipoprotein phenotype. The case control differences for plasma concentrations of major lipoproteins, glucose, insulin, and proinsulin-like molecules remained statistically significant after adjustment for the confounding effect of BMI (data not shown).

Relations of Insulin and Insulin Propeptides to Major Plasma Lipoproteins

Both basal and postload concentrations of insulin and proinsulin were strongly related to VLDL cholesterol and triglyceride levels in patients and controls (Table 2). In

Table 2. Spearman Rank Correlation Coefficients for the Relations of BMI and Basal Values and AUCs for OGTT Concentration Profiles for Glucose, Insulin, and Insulin Propeptides to Major Plasma Lipoproteins in Controls and Patients

	VLDL		LDL		HDL	
Parameter	C	TG	C	TG	C	TG
Controls						
Glucose						
Basal	.14	.14	.14	.02	−.06	.02
AUC	.25*	.28†	.16	.10	−.15	.12
Insulin						
Basal	.50‡	.48†	.25	.38*	−.38†	.20
AUC	.54‡	.53‡	.27	.27	−.19	.18
Proinsulin						
Basal	.51‡	.48‡	.39*	.36*	−.45*	−.03
AUC	.52†	.51‡	.40†	.36*	−.35*	.14
Des 31,32proinsulin						
Basal	.34*	.28	.17	.11	−.42*	−.15
AUC	.53‡	.46†	.33*	.28	−.35*	.09
BMI	.59‡	.65‡	.38*	.33*	−.25	.15
Patients						
Glucose						
Basal	.16	.13	.23	.22	−.23	−.06
AUC	.21	.20	.27*	.29*	−.16	.06
Insulin						
Basal	.57‡	.59‡	.10	.49‡	−.38†	.24
AUC	.52‡	.51‡	−.01	.40†	−.31*	.41†
Proinsulin						
Basal	.41†	.45‡	.08	.27*	−.23	.09
AUC	.48†	.46‡	.03	.18	−.15	.27*
Des 31,32proinsulin						
Basal	.41†	.48‡	−.11	.23	−.35†	.08
AUC	.50‡	.55‡	−.13	.23	−.31*	.28*
BMI	.26*	.29*	.28*	.32*	−.16	.20

Abbreviations: C, cholesterol; TG, triglycerides.
*$P < .05$.
†$P < .01$.
‡$P < .001$.

contrast, LDL cholesterol concentration was only significantly correlated with basal intact proinsulin and postload concentrations of proinsulin and des 31,32proinsulin in controls. LDL triglyceride level, on the other hand, was significantly related to basal insulin and proinsulin concentrations in both patients and controls, and to postload concentrations of insulin in patients and proinsulin in controls. Inverse associations were noted between basal insulin and HDL cholesterol levels in patients and controls, whereas proinsulin concentrations only correlated significantly with HDL cholesterol in controls. In contrast, des 31,32proinsulin was consistently inversely correlated with HDL cholesterol in all subjects. BMI was strongly correlated with VLDL lipid concentrations in controls, but was not related to HDL cholesterol level in either group (Table 2). When the confounding effect of age and BMI was taken into account by calculation of partial correlation coefficients, insulin and insulin propeptide relations to plasma lipoproteins weakened and were generally no longer statistically significant in the control group, but remained essentially unchanged in the patient group. Partial correlation coefficients between insulin, insulin propeptide, and VLDL lipid concentrations were reduced to .28 to .51 ($P < .05$ to .001) in the patient group and to .04 to .28 in controls (NS). Similarly, partial correlation coefficients with LDL lipids were no longer significant in the control group after adjustment for age and BMI, in contrast to the correlations of basal insulin ($r = .42, P < .001$) and insulin AUC ($r = .28$, $P < .05$) with LDL triglycerides among patients. Of note, the partial correlations with HDL cholesterol concentration were only slightly reduced in controls ($r = .25$ to $-.43$, $P < .1$ to .001) and patients ($r = -.28$ to $-.43$, $P < .05$ to .001).

Relations of Insulin and Insulin Propeptides to Subfractions of Apo B–Containing Lipoproteins

Associations of insulin and insulin propeptides to subfractions of apo B–containing lipoproteins were subsequently examined in greater detail in the patients (Tables 3 and 4). Basal and postload insulin and insulin propeptide concentrations were positively related to small (Sf 20-60) VLDL lipid and apo B levels, whereas no significant correlations were noted with the cholesteryl ester content per small VLDL particle (Table 3). This indicates that insulin and insulin propeptide concentrations are associated with the particle number rather than the composition of small VLDL. Correlation coefficients remained statistically significant ($r = .25$ to .50, $P < .05$ to .001) after controlling for age and BMI, except for the proinsulin correlation with small VLDL apo B. Similar correlations were also obtained between insulin, proinsulin, and des 31,32proinsulin levels and Sf > 100 and Sf 60-100 VLDL measurements (data not shown). Furthermore, basal and postload insulin concentrations correlated positively with the lipid and protein content in the IDL fraction (Table 4), whereas the corresponding associations for proinsulin and des 31,32proinsulin were not consistent. Insulin correlations were reduced after controlling for age and BMI but remained statistically

Table 3. Spearman Rank Correlation Coefficients for the Relations of BMI and Basal Values and AUCs for OGTT Concentration Profiles for Glucose, Insulin, and Insulin Propeptides to Small VLDL in Patients

	Small VLDL (Sf 20-60)			
Parameter	C	TG	Apo B	Mol CE
Glucose				
Basal	.18	.22	.12	−.11
AUC	.22	.26*	.15	−.03
Insulin				
Basal	.56‡	.60‡	.44‡	.23
AUC	.51‡	.57‡	.39†	.21
Proinsulin				
Basal	.41†	.42†	.29*	.24
AUC	.40†	.43‡	.33*	.26
Des 31,32proinsulin				
Basal	.38†	.39†	.26*	.13
AUC	.49‡	.53‡	.39†	.22
BMI	.26*	.31*	.14	.18

Abbreviations: Mol CE, number of molecules of cholesteryl esters per lipoprotein particle; TG, triglycerides; C, cholesterol.
*$P < .05$.
†$P < .01$.
‡$P < .001$.

Table 4. Spearman Rank Correlation Coefficients for the Relations of BMI and Basal Values and AUCs for OGTT Concentration Profiles for Glucose, Insulin, and Insulin Propeptides to IDL and LDL Subfractions in Patients

	IDL (d 1.006-1.019 kg/L)			Light LDL (d <1.019-1.040 kg/L)			Dense LDL (d 1.040-1.063 kg/L)		
Parameter	C	TG	Apo B	C	TG	Apo B	C	TG	Apo B
Glucose									
Basal	.23	.21	.21	.13	.14	.14	.34†	.30*	.41†
AUC	.33‡	.27*	.32*	.16	.27*	.16	.22	.18	.25
Insulin									
Basal	.45‡	.41†	.39†	−.19	.25	−.20	.04	.32*	.04
AUC	.40†	.42†	.34†	−.27*	.21	−.21	−.07	.27*	−.05
Proinsulin									
Basal	.33†	.28*	.17	−.15	.17	.13	.17	.18	.10
AUC	.23	.24	.12	−.24	.11	−.15	.10	.14	.02
Des 31,32 proinsulin									
Basal	.23	.21	.17	−.28*	−.15	−.25	.01	.16	.02
AUC	.31*	.33*	.28*	−.26	−.03	−.22	−.11	.11	−.08
BMI	.25	.25	.20	.16	.08	.25	.09	.23	.17

NOTE. Abbreviations are as in Tables 2 and 3.
*$P < .05$.
†$P < .01$.
‡$P < .001$.

significant ($r = .26$ to $.35$, $P < .05$ to $.01$) except for the insulin AUC correlations with IDL apo B, whereas the significant insulin propeptide correlations with IDL measurements were lost. It is notable that no consistent insulin and insulin propeptide relations were seen with the light and dense LDL fractions, with the exception of a weak positive correlation between basal and postload insulin concentrations and dense LDL triglyceride level, which remained essentially unchanged after controlling for age and BMI ($r = .21$ to $.31$, $P < .1$ to $.05$). Basal glucose concentration, on the other hand, correlated significantly with the lipid and protein content of the dense LDL fraction. This relation sustained the control for age and BMI ($r = .33$ to $.41$, $P < .01$). Similar correlations were also found between the glucose AUC and IDL measurements.

Separate analysis of NTG and HTG patients showed that the associations of insulin and insulin propeptides with apo B–containing lipoproteins observed in the entire patient group were mainly confined to NTG subjects (data not shown).

DISCUSSION

The aim of this study was to determine whether glucose intolerance, hyperinsulinemia, and insulin propeptides are implicated in determining a specific atherogenic lipoprotein pattern in nondiabetic men with premature coronary artery disease. Novel, specific assays for measuring proinsulin and des 31,32proinsulin, with a derived value for specific insulin, were used along with density-gradient ultracentrifugation procedures to quantify subfractions of apo B–containing lipoproteins. A substantial proportion of young male postinfarction patients were found to be glucose-intolerant, and basal and postload hyperinsulinemia were characteristic features irrespective of glucose tolerance category. Insulin resistance was present among the patients, particularly among HTG subjects. Basal and postload concentrations of proinsulin and des 31,32proinsulin were elevated to a similar extent in patients with normal or decreased oral glucose tolerance. However, the basal hyperinsulinemia and hyperinsulinemic responses found during an OGTT were due to "true" insulin and were not accounted for by a disproportionate increase in proinsulin or des 31,32proinsulin. Furthermore, HTG lipoprotein phenotypes with a low HDL cholesterol concentration dominated among the patients, and hyperinsulinemia was linked to hypertriglyceridemia and putatively atherogenic lipoprotein traits, such as increased particle numbers of small VLDL and IDL and triglyceride enrichment of LDL. In addition, fasting and postload glucose levels were associated with plasma concentrations of IDL and dense LDL.

On the basis of our findings, we propose that an abundance of small cholesteryl ester–rich VLDL and an elevated dense LDL triglyceride concentration be included in the specific lipoprotein phenotype that is linked to insulin resistance. The insulin resistance syndrome has previously been shown to consist of hypertension, central obesity, increased plasma plasminogen activator inhibitor-1 activity, hypertriglyceridemia, low HDL cholesterol concentration, increased IDL levels, and an LDL subclass pattern shifted toward smaller and denser particles.[14,31,32] The present results extend these previous observations to patients with premature coronary artery disease, and suggest that increased plasma levels of putatively atherogenic, small, cholesteryl ester–rich VLDL particles be added to the multiple lipoprotein disturbances of the insulin resistance syndrome. We have previously demonstrated that the cholesteryl ester content of small VLDL particles is related to the severity of coronary artery disease defined angiographically in young male survivors of myocardial infarction, particularly HTG patients, who also had increased plasma levels of dense, triglyceride-enriched LDL particles.[19] In the same vein, data from the Monitored Atherosclerosis Regression Study (MARS) study indicate that

triglyceride-rich lipoproteins (measured indirectly as apo C-III–heparin precipitate), with a predominance of smaller and denser VLDL subclasses, independently predict progression of angiographically defined mild to moderate atherosclerotic lesions in a group of patients treated with lovastatin.[33]

Hyperinsulinemia secondary to insulin resistance will increase hepatic VLDL triglyceride secretion rate and subsequently decrease VLDL catabolism with ensuing hypertriglyceridemia (for review, see Frayn[34]). Failure to suppress free fatty acid release from adipose tissue will enhance substrate availability in the liver, whereas impaired postprandial activation of lipoprotein lipase will reduce the potential for lipolysis.[34] However, it remains to be established whether insulin resistance causes hypertriglyceridemia or to some extent develops as part of the HTG state, or merely constitutes an integral part of a larger syndrome found in genetically predisposed individuals. It has been proposed that a single-gene trait is responsible for the small, dense LDL subclass pattern and related lipoprotein changes.[17,35,36]

In the present study, insulin propeptide concentrations correlated significantly with plasma levels of major lipoproteins and subfractions of apo B–containing lipoproteins. The strength of these associations was similar to those obtained for insulin. It has previously been reported that proinsulin is more strongly associated with well-established cardiovascular risk factors in type II diabetic subjects than is insulin.[7] Population data from the San Antonio Heart Study, including nondiabetic subjects, also demonstrate stronger relationships of proinsulin, as compared with insulin, to serum triglyceride concentration and systolic blood pressure.[8] In contrast to the San Antonio Heart Study, proinsulin concentration was significantly increased in coronary artery disease patients in the present study; however, the increase was proportionate to that of insulin. An explanation for the strong proinsulin correlations observed in the San Antonio Heart Study could be that proinsulin has an advantage over insulin in statistical models due to its lower intraindividual variability. Metabolic and biological actions also differ between insulin and insulin propeptides. Receptor-binding properties and metabolic effects of proinsulin and des 31,32proinsulin are considerably reduced as compared with insulin.[37] A more speculative hypothesis would be that retarded fetal growth, which is associated with glucose intolerance, hyperinsulinemia, increased concentrations of des 31,32proinsulin, increased fibrinogen and factor VII levels, and CHD, might be the underlying disturbance.[38-40]

Any possible influence of ongoing β-blocker medication on carbohydrate and lipoprotein metabolism could not be controlled for, since almost all patients were taking a cardioselective β-blocker as part of the routine postinfarction regimen. It is possible that the case control differences in basal and postload insulin and insulin propeptide levels might have been enhanced by β-blocker medication, whereas differences between subgroups of patients or between insulin and insulin propeptide correlations within these groups are less likely to depend on medication effects. Postload insulin concentrations in our patients were twofold to threefold higher than in controls, whereas considerably lower elevations of the insulin response to a glucose challenge have been noted after treatment with higher doses of metoprolol in previous studies (daily doses of metoprolol 200 mg *v* 50 to 100 mg in our patients, or atenolol 25 to 50 mg).[41,42] It has also been shown that short-term medication with metoprolol (100 mg daily) or atenolol (50 to 100 mg) does not alter insulin sensitivity.[42,43] The extent to which β-blocker treatment might have influenced lipoprotein metabolism, and LDL subclass distribution in particular, constitutes a conflicting issue.[44] Previous studies in our unit have failed to demonstrate a significant influence of short-term treatment with cardioselective β-blockers either on case control differences for plasma concentrations of major lipoprotein fractions and LDL subfractions or on lipoprotein correlations with coronary angiography scores.[11,45,46]

In summary, young nondiabetic male survivors of myocardial infarction are frequently glucose-intolerant. True hyperinsulinemia is present independently of whether glucose tolerance is normal or decreased, and is linked to hypertriglyceridemia and increased plasma levels of small VLDL and IDL particles and to LDL triglycerides. Insulin and insulin propeptides are proportionally elevated, and correlations with major plasma lipoproteins and subfractions of apo B–containing lipoproteins are generally similar for insulin, proinsulin, and des 31,32proinsulin. Multiple mechanisms are likely to be involved in determining this atherogenic lipoprotein phenotype. The fact that glucose intolerance and hyperinsulinemia have been identified as independent risk factors for future development of CHD[1,3,47,48] could be explained in part by the accompanying atherogenic lipoprotein profile.

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