Evidence for a Role of Tumor Necrosis Factor α in Disturbances of Triglyceride and Glucose Metabolism Predisposing to Coronary Heart Disease

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Elevated plasma levels of triglyceride-rich lipoproteins, a decreased high-density lipoprotein (HDL) cholesterol concentration, hyperinsulinemia, and impaired fibrinolytic function frequently aggregate in patients with premature coronary heart disease (CHD). Experimental studies suggest that the cytokine tumor necrosis factor α (TNFα) produced by adipocytes plays a part in the regulation of triglyceride and glucose metabolism. The present study examined whether TNFα is implicated in these metabolic and fibrinolytic disturbances in young postinfarction patients. TNFα levels were determined in two groups of young (age <45 years) male postinfarction patients (n = 92 and 60) and in matched, population-based control subjects (n = 63). Plasma TNFα was higher in patients than in controls (4.1 ± 1.6 *v* 2.5 ± 0.4 pg/mL, $P < .0001$). In hyperlipidemic patients, TNFα levels correlated significantly with the concentrations of very-low-density lipoprotein (VLDL) triglyceride and cholesterol and negatively with HDL cholesterol. Treatment with bezafibrate decreased VLDL triglycerides and increased HDL cholesterol, but did not affect TNFα levels. The TNFα concentration also correlated significantly with fasting glucose and proinsulin concentrations, as well as glucose and proinsulin levels after glucose ingestion. In contrast, no relations were found with the insulin level or degree of insulin resistance. The present results provide clinical evidence for a basic role of TNFα in hypertriglyceridemia, glucose intolerance, and the etiology of premature CHD.

TUMOR NECROSIS factor α (TNFα) is an inducible cytokine with a wide range of proinflammatory and immunoregulatory actions.[1,2] Macrophages and smooth muscle cells expressing TNFα are present in atherosclerotic plaques,[3,4] suggesting that TNFα may be involved in atherogenesis.

TNFα also has potent metabolic effects, and it was originally identified as the factor responsible for induction of hypertriglyceridemia in bacterially infected animals.[5] This effect of TNFα was subsequently shown to be due to an increased synthesis of triglycerides in the liver[6] and an inhibition of lipoprotein lipase (LPL).[7] More recently, TNFα has been implicated in the etiology of insulin resistance in obesity.[8] This concept was based on observations made in genetically obese mice that have an increased expression of TNFα in adipose tissue and respond with improved insulin sensitivity to infusion of a TNFα-blocking protein.[9] The effect of TNFα on insulin sensitivity appears to be mediated by inhibition of insulin receptor phosphorylation.[10,11] Recent studies have demonstrated increased adipose and muscle tissue expression of TNFα also in human obesity and insulin resistance.[12-14]

Hypertriglyceridemia and reduced high-density lipoprotein (HDL) cholesterol levels accompanied by hyperinsulinemia and impaired fibrinolytic capacity are common findings in patients with premature coronary heart disease (CHD).[15,16] The present sample of young postinfarction patients and population-based controls were used to investigate if TNFα is involved in these metabolic disturbances and thus implicated in the etiology of CHD.

SUBJECTS AND METHODS

Study Population and Protocol

A total of 152 men with a first myocardial infarction before the age of 45 years were investigated after provision of informed consent. The patients had initially been admitted to the 10 hospitals in Stockholm County with coronary care or intensive care units between January 1985 and October 1990. Patients with known diabetes mellitus, severely impaired renal function, or other concomitant diseases were excluded. Initial metabolic studies were performed 4 to 6 months after the infarction to allow acute-phase reactions due to myocardial damage to subside. No patient was taking lipid-lowering drugs at the time of the initial metabolic investigation, but all had received instruction on a lipid-lowering diet by a dietitian at an outpatient clinic visit 6 weeks after the initial admission. The patients were originally recruited for inclusion into two different studies. The first patient sample consisted of 92 men recruited between January 1985 and December 1988 who entered the 5-year Bezafibrate Coronary Atherosclerosis Intervention Trial (BECAIT). This study included patients with serum cholesterol between 5.2 and 10.0 mmol/L and/or serum triglycerides between 1.6 and 8.0 mmol/L and angiographic signs of coronary atherosclerosis. The second study population consisted of 60 men recruited consecutively between April 1989 and October 1990 to participate in a detailed evaluation of abnormalities in lipoprotein and glucose metabolism in young postinfarction patients. Sixty-three healthy men with an age distribution similar to that of the latter patient group were also recruited concurrently with these patients by random selection of individuals born between 1947 and 1956 from a register of all permanent residents in Stockholm County. Detailed descriptions of both patient populations and the controls have been published previously.[17-19]

Laboratory Methods and Procedures

Blood samples for lipoprotein analyses and hemostatic tests were taken between 8 and 9 AM after 12 hours of fasting, during which time

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smokers were asked to refrain from smoking. All subjects were free from symptoms of infectious disease at the time of blood sampling. Detailed descriptions of the preparation of serum and plasma samples have been published previously.[17-19] On a separate visit after 12 hours of fasting, glucose was ingested at a dose of 1.75 g/kg body weight in 150 to 200 mL water flavored with lemon extract.[20] Venous blood samples for determination of blood glucose and immunoreactive insulin (BECAIT study), respectively, for blood glucose, insulin, and insulin propeptides (unselected patients and controls) were obtained before and 15, 30, 45, 60, 90, and 120 minutes after glucose intake, with the subjects remaining semirecumbent throughout the test.

The major plasma lipoproteins (very–low-density lipoprotein [VLDL], low-density lipoprotein [LDL], and HDL were determined by a combination of preparative ultracentrifugation, precipitation of apolipoprotein B–containing lipoproteins, and lipid analyses.[21] Blood glucose levels were measured by the glucose oxidase method (Kodak Ektachem; Eastman Kodak, Rochester, NY). Plasma insulin was determined by an in-house double-antibody radioimmunoassay (BECAIT study) or by a radioimmunoassay with polyclonal antisera supplied by Guildhay (Guilford, UK). Immunoradiometric assays were used to measure the levels of intact proinsulin and des 31,32 proinsulin.[22] Intact proinsulin was assayed using murine monoclonal antibodies A6 and 3B1 (Serono Diagnostics, Woking, UK). Murine monoclonal antibody 3B1 and antibody PEP-001 (Novo Nordisk, Baagsvard, Denmark) were used for the two-site des 31,32 proinsulin assay. True insulin was obtained from the insulin radioimmunoassay by subtraction of intact proinsulin and corrected des 31,32 proinsulin. Insulin resistance was calculated from fasting blood glucose and plasma insulin concentrations by the homeostasis model assessment (HOMA) method.[23] Plasminogen activator inhibitor-1 (PAI-1) activity was determined by adding a certain amount of tissue plasminogen activator (t-PA) to diluted plasma and measuring residual t-PA activity using an amidolytic method with chromogenic substrate (Spectrolyse fibrin; Biopool, Umeå, Sweden).[24] The levels of the acute-phase reactants orosomucoid and haptoglobin were measured by electroimmunoassay.[25] Haptoglobin levels were only measured in 47 BECAIT patients at baseline and in 74 patients at the end of this study.

Plasma and serum TNFα levels were measured using an enzyme-linked immunosorbent assay (ELISA) for human TNFα (Quantikine; R&D Systems, Minneapolis, MN). This is a newly developed, sensitive sandwich ELISA with a lower detection limit of 0.5 pg/mL. The intraassay coefficient of variation for analysis of TNFα in serum is less than 8%, and the mean recovery of the assay is 95% for serum and 96% for EDTA plasma. The correlation coefficient for duplicate determinations on the same sample was .93 (n = 92) in the present study. There was no difference in the mean level of TNFα in serum samples obtained at baseline of the BECAIT study (4.70 ± 1.43 pg/mL) and samples obtained at the end of this study (4.79 ± 1.36 pg/mL), suggesting that serum samples could be stored at −70°C for at least 5 years without significant degradation of TNFα.

Coronary Angiography

Selective coronary angiography was performed about 6 months after the acute event. The severity of coronary lesions was determined by computer-assisted evaluation in BECAIT patients using the cardiovascular measurement system.[26] The minimum lumen diameter (which reflects focal atherosclerosis) was measured at the most severe atherosclerotic lesions that reduced the lumen diameter by at least 20% in each of 15 coronary artery segments defined according to American Heart Association recommendations.[27] The mean segment diameter was calculated in all coronary segments irrespective of the presence of visually detectable atherosclerosis, as an indicator of diffuse and focal atherosclerosis. These measurements were taken only in unoccluded segments. The mean minimum lumen diameter and mean segment diameter for all evaluated segments were used as summary estimates of the severity of coronary artery disease. Visual semiquantitative classifications of diffuse atherosclerosis and the number and severity of distinct stenoses in identical segments were made in the unselected patient group. Detailed descriptions of these analyses have been published previously.[19,28]

Statistical Analysis

Conventional methods were used for calculation of the mean ± SD. Coefficients of skewness were calculated to test deviation from the normal distribution. Differences in continuous variables between groups were tested by ANOVA and Scheffé's F test. Distributions of categorical data were compared using the χ^2 test with Yates' correction. Spearman rank correlation coefficients were calculated to estimate relations between biochemical variables or between biochemical variables and angiographic scores.

RESULTS

TNFα Levels in Postinfarction Patients and Controls

Characteristics of the two patient populations and the controls are listed in Table 1. Patients were characterized by a higher body mass index (BMI), and there were more former smokers in these groups. A diagnosis of hypertension was also more common among patients. Furthermore, patients had higher VLDL and LDL cholesterol and lower HDL cholesterol concentrations than controls. VLDL triglycerides were considerably elevated among patients who were additionally characterized by basal and postload hyperglycemia and high plasma PAI-1 activity.

The plasma level of TNFα was significantly higher in the unselected patients than in the age-matched control group (4.1 ± 1.6 *v* 2.5 ± 0.4 pg/mL, $P < .0001$; Fig 1). The TNFα

Table 1. Characteristics of the Study Groups

Characteristic	BECAIT Patients (n = 92)	Unselected Postinfarction Patients (n = 60)	Controls (n = 63)
Age (yr)	40.9 ± 3.1	39.6 ± 4.0	39.7 ± 2.6
BMI (kg/m²)	27.2 ± 3.89	28.3 ± 4.1†	24.4 ± 2.9
Smoking history (n)			
Nonsmoker	12	6	33
Former smoker	58	35†	8
Present smoker	22	19	16
Hypertension (%)	20	21†	3
Cholesterol (mmol/L)			
VLDL	1.14 ± 0.68	1.03 ± 0.94†	0.52 ± 0.56
LDL	4.62 ± 1.00	4.32 ± 1.07†	3.59 ± 0.91
HDL	1.08 ± 0.21	0.91 ± 0.23†	1.12 ± 0.31
Triglycerides (mmol/L)			
VLDL	1.88 ± 1.18	2.35 ± 2.22†	1.19 ± 1.39
LDL	0.42 ± 0.11	0.46 ± 0.13†	0.33 ± 0.11
HDL	0.15 ± 0.04	0.13 ± 0.04*	0.11 ± 0.04
Glucose (mmol/L)			
Basal	5.4 ± 1.3	5.3 ± 1.1†	4.6 ± 0.4
AUC	9,222 ± 2,031	9,749 ± 2,883†	7,905 ± 1,576
PAI-1 (U/mL)	27.3 ± 16.9	30.0 ± 29.0†	13.6 ± 10.7

NOTE. Values are the mean ± SD. Significance testing (unpaired *t* test and χ^2 test) was only performed between unselected postinfarction patients and controls.

Abbreviation: AUC, area under the curve.

*$P < .01$.

†$P < .001$.

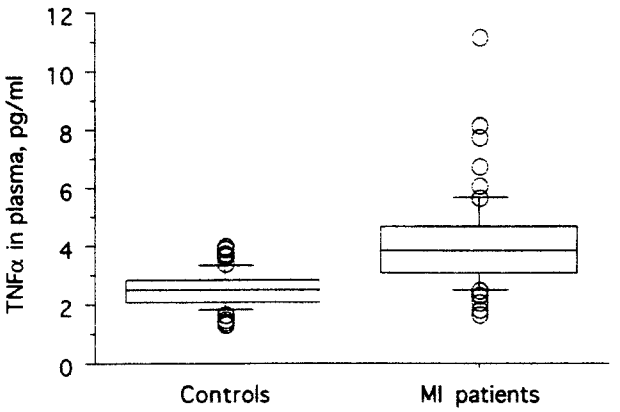

Fig 1. Box plot demonstrating the median and 10th, 25th, 75th, and 90th percentiles for plasma TNFα levels in 60 unselected young postinfarction (MI) patients and 63 age-matched healthy controls. (○) Individual values < the 10th and > the 90th percentiles.

level in BECAIT patients (4.7 ± 1.4 pg/mL) was analyzed in serum and therefore cannot be compared directly with that in the other two groups.

There were no relations between BMI and TNFα levels in any of the groups (Table 2). Since TNFα is an important inflammatory mediator, we performed an analysis of its relation to the acute-phase reactants orosomucoid and haptoglobin; these analyses were performed only in BECAIT patients. The mean serum levels of orosomucoid and haptoglobin in samples taken at baseline of the BECAIT study were 0.92 ± 0.20 and 1.20 ± 0.50 g/L, respectively. There were no relations between TNFα levels and orosomucoid ($r = .08$) or haptoglobin ($r = -.02$), whereas a significant correlation existed between orosomucoid and haptoglobin ($r = .37$, $P = .01$). Analyses of TNFα and acute-phase reactants were also performed at the end of the BECAIT study. Again, there were no relations between TNFα levels and orosomucoid ($r = -.17$) or haptoglobin ($r = -.03$).

There were no differences in TNFα levels between nonsmokers, former smokers, and smokers nor between hypertensives and nonhypertensives in any of the groups.

Relations to Lipoprotein Lipids

An inverse relation between TNFα and HDL cholesterol was seen in both the patients and the controls (Table 3). Among unselected patients, there was also an inverse relation between TNFα and LDL cholesterol. In BECAIT patients, significant correlations were found between TNFα and VLDL triglycerides and cholesterol. The corresponding correlation did not attain statistical significance in unselected patients and controls.

Table 2. TNFα Concentrations in Subjects Grouped According to BMI

Group	BMI (kg/m²)		
	<25	25-30	>30
BECAIT patients	4.6 ± 1.4	4.6 ± 1.4	4.9 ± 1.4
Unselected postinfarction patients	4.1 ± 1.2	4.2 ± 1.8	3.6 ± 1.2
Controls	2.5 ± 0.6	2.5 ± 0.6	2.6 ± 0.9

NOTE. Values are the mean ± SD.

Since BECAIT patients represented a selected subgroup of dyslipidemic postinfarction patients with angiographically demonstrable coronary atherosclerosis, a second analysis was performed among the unselected postinfarction patients who would have fulfilled the inclusion criteria of the BECAIT study. Since our primary interest was to analyze the relation between TNFα and components of the insulin resistance syndrome, we also excluded patients with isolated hypercholesterolemia (who constituted <4% of the BECAIT population). Forty-seven patients remained after normolipidemic and type IIA hypercholesterolemic patients and patients without lesions on coronary angiography were excluded. In this subgroup, significant correlations existed between TNFα and VLDL triglycerides ($r = .44$, $P < .01$), VLDL cholesterol ($r = .43$, $P \leq .01$), and HDL cholesterol ($r = -.37$, $P < .05$). Compared with the 17 excluded subjects, the patients in this subgroup had higher levels of LDL triglycerides (0.49 *v* 0.39 mmol/L, $P < .05$), VLDL cholesterol (1.27 *v* 0.42 mmol/L, $P < .005$) and triglycerides (2.95 *v* 0.85 mmol/L, $P < .001$), and HDL triglycerides (0.14 *v* 0.11 mmol/L, $P < .05$) and a higher BMI (29.1 *v* 26.3, $P < .05$), whereas there were no significant differences in the levels of the remaining lipoprotein lipids and TNFα (4.0 *v* 4.4 pg/mL).

Relations to Glucose-Insulin Homeostasis

TNFα correlated significantly with the glucose area under the curve during the oral glucose tolerance test in BECAIT patients ($r = .25$, $P < .05$) and in the hypertriglyceridemic subgroup of the unselected patient sample ($r = .37$, $P < .05$), but not in the controls ($r = .06$, NS). The relations of TNFα to insulin and insulin propeptides were also analyzed in the subgroup of hypertriglyceridemic men from the unselected patient group, in whom significant correlations between TNFα and triglyceride-rich lipoproteins had been found, and in the controls. Basal and postload glucose, insulin, proinsulin, des 31,32 proinsulin, and C-peptide levels were higher in these patients than in the control group (data not shown). There was a significant correlation between TNFα and basal levels of blood glucose, proinsulin, des 31,32 proinsulin, and C-peptide in the patient group (Table 4). Similar correlations were found at 120 minutes, and in most instances at 15 minutes, after glucose ingestion. In contrast, there were no relations between TNFα and glucose/insulin variables among the controls (data not shown). Of note, TNFα concentrations were not correlated with insulin concentrations in the patients or with the degree of insulin resistance assessed by the HOMA method ($r = .10$, NS). TNFα was also significantly related to glucose and proinsulin levels in the complete unselected postinfarction group (n = 60), but the correlation coefficients were generally lower than in the hyperlipidemic subgroup ($r = .30$, $P < .05$, $r = .37$, $P < .005$, and $r = .31$, $P < .05$) for fasting glucose and des 31,32 proinsulin and plasma glucose 15 minutes after glucose ingestion, respectively.

Effect of Triglyceride Reduction

In patients treated with bezafibrate (200 mg three times daily) for 24 months, VLDL cholesterol and triglyceride levels decreased by 35% and 31%, respectively, and HDL cholesterol increased by 9%. No significant changes in lipoprotein lipids occurred in the placebo-treated group. The serum level of TNFα

Table 3. Correlation Coefficients Between TNFα and Lipoprotein Lipid Concentrations

Group	VLDL		LDL		HDL	
	Chol	TG	Chol	TG	Chol	TG
Controls (n = 63)	.24	.20	−.02	.12	−.32*	.04
Unselected postinfarction patients (n = 60)	.21	.19	−.29*	.10	−.27*	.16
BECAIT patients						
Baseline (n = 92)	.37‡	.40‡	.00	.18	−.28†	.20
Placebo group at 2 years (n = 45)	.54†	.53†	−.03	.15	−.36*	.21
Bezafibrate group at 2 years (n = 47)	.23	.18	.05	.20	−.13	−.17

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

remained unchanged in both the bezafibrate (+2.8%) and the placebo (−2.7%) groups, and there was a close correlation between TNFα levels at baseline and 24 months after randomization ($r = .65$, $P < .005$). Significant correlations between TNFα and lipoprotein lipids at baseline remained at 24 months in the placebo-treated group, but did not persist in the bezafibrate-treated group (Table 3).

No Link Between the TNFα Level and the Severity of Coronary Atherosclerosis

There were no relations between the TNFα level and the severity of coronary atherosclerosis (focal or diffuse) in the two patient groups.

DISCUSSION

Hypertriglyceridemia accompanied by a low HDL cholesterol concentration, basal and postload hyperinsulinemia, and an impaired fibrinolytic capacity is a common finding in patients with premature CHD.[15,16] These abnormalities often occur as part of a metabolic syndrome also including obesity and hypertension.[29] More recently, overproduction of TNFα in adipose tissue has been associated with insulin resistance, and it has been proposed that TNFα is the biological mediator of insulin resistance in obesity.[8] Against this background, we examined the hypothesis that TNFα is involved in the metabolic abnormalities appearing in a major subset of young postinfarction patients. TNFα levels were found to be increased about twofold in the entire patient group as compared with age-matched, population-based controls. Among hyperlipidemic postinfarction patients, high TNFα was associated with hypertriglyceridemia, low HDL cholesterol, and perturbed glucose metabolism. TNFα levels were also elevated in normolipidemic postinfarction patients, but did not correlate with lipoprotein lipids. Taken together, these observations demonstrate increased levels of TNFα in patients with early-onset CHD and suggest that TNFα may play a role in the metabolic disturbances found in many of these patients. However, it must be emphasized that the data should be interpreted cautiously, as a substantial proportion of the study groups were small and some of the results were generated by post hoc analysis. Moreover, conclusions regarding the possible pathophysiological role of TNFα in the two groups are complicated by the considerable metabolic differences that exist.

Table 4. Correlation Coefficients Between TNFα and Basal and Postload Glucose/Insulin Concentrations in Unselected Postinfarction Patients With a Hypertriglyceridemic Lipoprotein Phenotype (n = 47)

Parameter	Time Point		
	0 min	15 min	120 min
Glucose	.33*	.44†	.38*
Insulin	.12	.07	.18
Proinsulin	.31*	.28	.31*
Des 31,32 proinsulin	.38*	.30*	.36*
C-peptide	.32*	.22	.37*

*$P < .05$.
†$P < .01$.

Because TNFα is a cytokine, plasma levels increase in response to tissue injury such as myocardial infarction and infection. To avoid this confounding factor, all TNFα and metabolic determinations were performed 6 months after the acute event, and symptoms of infectious disease precluded blood sampling. The lack of a correlation between TNFα and the acute-phase reactants orosomucoid and haptoglobin also argues against the possibility that the associations between TNFα and metabolic variables observed in this study are explained by a general inflammatory reaction. We did not determine acute-phase reactants in the control population in this study. However, we have previously shown that young postinfarction patients have higher serum levels of orosomucoid than age-matched controls.[30] Accordingly, it is possible that the difference in TNFα levels between postinfarction patients and controls is, in part, due to increased inflammatory activity in the latter group. Moreover, although there were no differences in TNFα levels between smokers and nonsmokers or between hypertensives and nonhypertensives, it cannot be excluded that TNFα levels among postinfarction patients are influenced by these risk factors.

What, then, is the link between TNFα and the metabolic disturbances analyzed in this study? One possible explanation could be that hypertriglyceridemia itself stimulates the release of TNFα. However, the fact that bezafibrate treatment did not affect TNFα levels despite a 30% decrease in serum triglycerides argues against this interpretation. Another possibility is that the metabolic abnormalities and the increase in TNFα both occur in response to a yet unidentified factor. However, there are no experimental data supporting this concept.

Adipose tissue expression of TNFα is increased in obese

mice and in humans with insulin resistance,[9] and weight reduction in obese subjects results in decreased adipose expression of TNFα and improved insulin sensitivity.[12,13] Against this background, it has been proposed that obesity causes insulin resistance by increasing TNFα synthesis in adipose tissue and that this mechanism represents a defense against tissue fat overload.[8] Interestingly, TNFα did not correlate with BMI in young postinfarction patients or in the controls, and TNFα levels were increased also in lean postinfarction patients. Hence, it seems likely that factors other than obesity are responsible for the increased TNFα levels in these patients.

No relationships were noted in the present study between TNFα levels and plasma insulin concentrations or the degree of insulin resistance as measured by the HOMA method. This is interesting in view of experimental studies indicating that localized production of TNFα in adipose tissue and muscle can induce resistance to insulin-stimulated glucose uptake, probably via a reduction in insulin receptor tyrosine kinase activity.[10,12,14] However, the insulin resistance data must be interpreted with caution, because the HOMA method provides a crude measure of insulin resistance. Proinsulin-like molecules might, in fact, be more stable markers for insulin resistance than plasma concentrations of insulin. The positive relations between TNFα and proinsulin or des 31,32 proinsulin concentrations could thus be interpreted to suggest an association between elevated TNFα levels and insulin resistance among the young postinfarction patients. Reduced glucose uptake has been the only index of insulin resistance thus far evaluated with respect to TNFα, but the sensitivity of other insulin-modulated processes could also be affected. LPL activity increases in response to insulin,[31] and it is noteworthy that an inverse correlation has been reported between LPL activity and TNFα.[13] Resistance to suppression of adipose tissue lipolysis by insulin could be another effect of TNFα, especially in view of the localized tissue actions of the cytokine. Such an effect would increase the rate of adipose tissue lipolysis and the rate of release of nonesterified fatty acids (NEFAs) into the circulation.[32] NEFA supply to the liver is one of the principal determinants of the rate of hepatic triglyceride synthesis.[33] Also, NEFAs can compete with glucose as a fuel.[34,35] In the present study, the strongest associations between TNFα and VLDL triglyceride levels and between TNFα and glucose concentrations were seen in patients with a hypertriglyceridemic phenotype, as might be expected if increased NEFA flux was an important common factor. Further studies of the relation between TNFα and NEFA metabolism will be needed to confirm whether this is in fact the case. The association between TNFα and plasma proinsulin levels could be related, in part, to the compensatory pancreatic insulin response to TNFα-induced insulin resistance, but the lack of an association of TNFα with the plasma insulin level and insulin resistance suggests that other factors may be operating. One possibility is that in individuals with high TNFα levels, hypertriglyceridemia, and hyperglycemia, there is some disruption in the processing of proinsulin in the pancreatic β cell, leading to an increased proportion of proinsulin released into the circulation.

It appears from this study that elevated TNFα levels are associated with atherogenic metabolic disturbances in men with premature CHD. A likely interpretation of the present data is that TNFα is implicated both in determining a hypertriglyceridemic lipoprotein phenotype and in the etiology of premature CHD. The restrictions of a clinical case-control study notwithstanding, no support was obtained for the notion that TNFα plays a major basic role in the prominent insulin resistance component in this group of coronary patients. Further studies are needed to explore whether TNFα and/or hypertriglyceridemia may perturb pancreatic β-cell function.

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REFERENCES

1. Old LJ: Tumor necrosis factor (TNF). Science 230:630-633, 1985

2. Beutler B, Cerami A: The biology of cachectin/TNF—A primary mediator of the host response. Annu Rev Immunol 7:625-655, 1989

3. Barath P, Fishbein M, Cao J, et al: Detection and localization of tumour necrosis factor in human atheroma. Am J Pathol 65:297-302, 1990

4. Tipping PG, Hancock WW: Production of tumor necrosis factor and interleukin-1 by macrophages from atheromatous plaque. Am J Pathol 142:1721-1728, 1993

5. Rouzer CA, Cerami A: Hypertriglyceridemia associated with *Trypanosoma brucei brucei* infection in rabbits: Role of defective triglyceride removal. Mol Biochem Parasitol 2:31-38, 1980

6. Feingold KR, Serio MK, Adi S, et al: Tumor necrosis factor stimulates hepatic lipid synthesis and secretion. Endocrinology 124:2336-2342, 1989

7. Kawakami M, Cerami A: Studies of endotoxin-induced decrease in lipoprotein lipase activity. J Exp Med 154:631-639, 1981

8. Hotamisligil GS, Spiegelman BM: Tumor necrosis factor α: A key component of the obesity-diabetes link. Diabetes 43:1271-1278, 1994

9. Hotamisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factor-α: Direct role in obesity-linked insulin resistance. Science 259:87-91, 1993

10. Hotamisligil GS, Budavari A, Murray D, et al: Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-α. J Clin Invest 94:1543-1549, 1994

11. Hotamisligil GS, Murray DL, Choy LN, et al: Tumor necrosis factor α inhibits signaling from the insulin receptor. Proc Natl Acad Sci USA 91:4854-4858, 1994

12. Hotamisligil GS, Arner P, Caro JF, et al: Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance. J Clin Invest 95:2409-2415, 1995

13. Kern PA, Saghizadeh M, Ong JM, et al: The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest 95:2111-2119, 1995

14. Saghizadeh M, Ong JM, Garvey WT, et al: The expression of TNFα by human muscle. Relationship to insulin resistance. J Clin Invest 97:1111-1116, 1996

15. Båvenholm P, Malmberg K, Efendic S, et al: Hyperinsulinaemia, hypertriglyceridaemia and impaired fibrinolytic function: Concordant

abnormalities associated with myocardial infarction at young age. Nutr Metab Cardiovasc Dis 3:28-37, 1993

16. Malmberg K, Båvenholm P, Hamsten A: Clinical and biochemical factors associated with prognosis after myocardial infarction at young age. J Am Coll Cardiol 24:592-599, 1994

17. Tornvall P, Båveholm P, Landou C, et al: Relationship of plasma levels and composition of apolipoprotein B–containing lipoproteins to angiographically defined coronary artery disease in young patients with myocardial infarction. Circulation 88:2180-2189, 1993

18. de Faire U, Ericsson C-G, Hamsten A, et al: Design features of a five-year Bezafibrate Coronary Atherosclerosis Intervention Trial (BECAIT). Drugs Exp Clin Res 21:105-124, 1995

19. Ericsson CG, Hamsten A, Nilsson J, et al: Angiographic assessment of the effects of bezafibrate on progression of coronary artery disease in young male postinfarction patients. Lancet 347:849-853, 1996

20. Reaven TG, Bernstein R, Davis B, et al: Non-ketotic diabetes mellitus: Insulin deficiency or insulin resistance? Am J Med 60:80-88, 1976

21. Carlson K: Lipoprotein fractionation. J Clin Pathol 5:32-37, 1973

22. Proundler AJ, Godsland IF, Stevenson JC: Insulin propeptides in conditions associated with insulin resistance in humans and their relevance to insulin measurements. Metabolism 43:446-449, 1995

23. Matthews DR, Hosher JP, Rudenski AS, et al: Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412-419, 1985

24. Chmielewska J, Rånby M, Wiman B: Evidence for a rapid inhibitor to tissue plasminogen activator in plasma. Thromb Res 31:427-436, 1983

25. Laurell CB: Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal Biochem 15:45-52, 1966

26. Reiber JHC, Serruys PW: Quantitative coronary angiography, in Reiber JHC (ed): Developments in Cardiovascular Medicine. Dordrecht, The Netherlands, Kluwer Academic, 1991

27. Austen WG, Edwards JE, Frye RL, et al: A reporting system on patients evaluated for coronary artery disease. Circulation 51:5-40, 1975

28. Hamsten A, Walldius G, Szamosi A, et al: Relationship of angiographically defined coronary artery disease to serum lipoproteins in young survivors of myocardial infarction. Circulation 73:1097-1110, 1986

29. Reaven GM: Banting Lecture 1988: Role of insulin resistance in human disease. Diabetes 37:1595-1607, 1988

30. Hamsten A, Blombäck M, Wiman B, et al: Haemostatic function in myocardial infarction. Br Heart J 55:58-66, 1986

31. Sadur C, Eckel R: Insulin stimulation of adipose tissue lipoprotein lipase. J Clin Invest 69:1119-1125, 1982

32. Coppack SW, Evans RD, Fisher RM, et al: Adipose tissue metabolism in obesity: Lipase action before and after a mixed meal. Metabolism 41:264-272, 1992

33. Reaven G, Mondon C: Effect of in vivo plasma insulin levels on the relationship between perfusate free fatty acid concentration and triglyceride secretion by perfused rat livers. Horm Metab Res 16:230-232, 1984

34. Randle P, Garland P, Hales C, et al: The glucose fatty acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1:785-789, 1963

35. Groop LC, Saloranta C, Shank M, et al: The role of free fatty acid metabolism in the pathogenesis of insulin resistance in obesity and non–insulin-dependent diabetes mellitus. J Clin Endocrinol Metab 72:96-107, 1991