European Heart Journal (2002) **23,** 376–383
doi:10.1053/euhj.2001.2805, available online at http://www.idealibrary.com on IDEAL®

Plasma tumour necrosis factor-α and early carotid atherosclerosis in healthy middle-aged men

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Aims Tumour necrosis factor-α (TNF-α) is a proinflammatory cytokine, which is implicated in some metabolic disorders and may play a role in the development of cardiovascular disease. We examined whether plasma TNF-α is related to established cardiovascular risk indicators, plasma levels of soluble cellular adhesion molecules and carotid artery intima–media thickness determined by ultrasound examination in a population-based cohort of 96 healthy 50-year-old men.

Methods and Results TNF-α and cellular adhesion molecules were measured with enzyme-linked immunosorbent assays. Plasma TNF-α concentration was associated with systolic and diastolic blood pressure, degrees of alimentary lipaemia, plasma very low density lipoprotein triglyceride, low density lipoprotein (LDL) cholesterol concentrations and peak LDL particle size. Two indices of insulin resistance as well as all soluble cellular adhesion molecules correlated positively with TNF-α. The plasma TNF-α concentration was associated with common carotid intima–media thickness in univariate analysis. In contrast, soluble E-selectin and postprandial triglycerides, but not TNF-α, were independent determinants of common carotid intima–media thickness.

Conclusion The plasma TNF-α concentration is associated with degrees of early atherosclerosis and correlates with metabolic and cellular perturbations that are considered important for the vascular process.
(Eur Heart J, 2002; 23: 376–383, doi:10.1053/euhj.2001.2805)

Key Words: Atherosclerosis, common carotid intima–media thickness, tumour necrosis factor-α, intercellular adhesion molecule-1, vascular adhesion molecule-1, E-selectin.

See page 345, doi: 10.1053/euhj.2001.2905 for the Editorial comment on this article

Introduction

Tumour necrosis factor-α (TNF-α) is a cytokine with a wide range of proinflammatory activities[1,2]. It is primarily produced by monocytes/macrophages[3], although significant amounts are also secreted by several other cell types. Disturbances in the TNF-α metabolism have been implicated in metabolic disorders, such as obesity and insulin resistance[4,5], indicating that perturbations of TNF-α metabolism may affect the onset of non-insulin-dependent diabetes mellitus and play a role in the development of cardiovascular disorders. Indeed, increased plasma concentrations of TNF-α have been found in patients with premature coronary artery disease[6]. However, it remains unclear whether elevated serum TNF-α in patients with manifest atherosclerosis derives from atherosclerotic plaques or from non-vascular sources. Be that as it may, the primary proinflammatory cytokine TNF-α, in turn, elicits the expression of the messenger cytokine interleukin-6, which induces expression of hepatic genes encoding acute-phase reactants, as well as the production of other effector molecules in the inflammatory response, such as cellular adhesion molecules for leukocytes[7]. Adhesion of circulating leukocytes to endothelial cells with ensuing transendothelial migration is considered an important early step in atherogenesis[8], and increased

Revision submitted 1 May 2001, accepted 1 June 2001, and published online 19 September 2001.

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0195-668X/02/050376+08 $35.00/0

expression of cellular adhesion molecules may accordingly be one mechanism by which TNF-α is implicated in atherothrombotic disease. That the plasma concentration of the soluble form of at least one cellular adhesion molecule, intercellular adhesion molecule type-1 (sICAM-1), is in fact elevated several years before the occurrence of clinical manifestations of coronary artery disease was recently demonstrated in two prospective epidemiological studies[9,10], and pathological studies have shown increased cellular adhesion molecule expression in several components of the atherosclerotic plaque[11–14]. Furthermore, the plasma concentrations of one or more soluble cellular adhesion molecules have been found to correlate with carotid intima–media thickness[9,15–17], a marker of early atherosclerosis, and with severity of angiographically assessed atherosclerosis of the abdominal aorta and pelvic and leg arteries[18] in cross-sectional studies of various populations, but study results in relation to measures of atherosclerosis have been far from consistent.

The present study was conducted to explore the relationships of plasma TNF-α to established clinical and metabolic risk indicators for coronary artery disease and to plasma levels of soluble cellular adhesion molecules in a population-based cohort of healthy 50-year-old men. A second objective was to examine the associations of TNF-α and soluble cellular adhesion molecules with carotid intima–media thickness, along with a wide range of other biochemical and clinical risk markers, the hypothesis being that the proinflammatory cytokine TNF-α may influence the atherosclerotic process both by causing metabolic perturbations and by increasing the expression of cellular adhesion molecules. The clinical characteristics and the relationships of plasma concentrations of fasting and postprandial lipoproteins to common carotid intima–media thickness observed in this cohort have been reported[19].

Methods

Subjects

A total of 96 50-year-old Caucasian men living in the northern parts of the county of Stockholm participated in the study. They were randomly selected from a registry comprising all permanent residents. Inclusion criteria, in addition to male sex and age of 50 years, were North European or North American descent, the presence of an apolipoprotein E3/E3 genotype and acquisition of technically satisfactory carotid ultrasound images. Exclusion criteria were chronic disease of any kind, a history of coronary artery disease or arterial thromboembolic disease, familial hypercholesterolaemia, body mass index $>32\ kg\ .\ m^{-2}$, alcohol abuse or psychiatric disorders that would interfere with compliance, and participation in other ongoing studies. The recruitment procedures and the representativeness of the cohort have been described[19]. The Ethics Committee of the Karolinska Hospital approved the study, and all subjects gave their informed consent to participate.

Study protocol

Blood samples were first obtained in the early morning after an overnight fast. Participants then ingested a mixed meal. The test meal consisted of pasta, boiled drawn chicken breast meat, green peas and mayonnaise[19]. The mayonnaise was prepared from soybean oil (Karlshamns Oils & Fats AB, Karlshamn, Sweden). The total energy content of the meal was 1000 kcal with 60 E% from fat, 13 E% from protein and 27 E% from carbohydrate. This corresponds to a fat load of approximately 65 g. Postprandial blood samples were drawn every hour for determination of plasma triglycerides and after 3 and 6 h for determination of apo B-48 and apo B-100 in Svedberg flotation rate (Sf) >400, Sf 60-400, Sf 20-60 and Sf 12-20 lipoprotein fractions. Water and tea, but no other food and no smoking, were allowed during the test. A carotid artery ultrasound examination was performed in connection with the oral fat load.

Measurements of TNF-α and circulating cellular adhesion molecules

TNF-α, sICAM-1, soluble vascular cell adhesion molecule type-1 (sVCAM-1) and soluble E-selectin were measured in duplicate in citrate plasma samples with use of commercially available enzyme-linked immunosorbent assays (ELISAs), based on the quantitative sandwich enzyme immunoassay technique, according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, U.S.A.).

Plasma lipids and lipoproteins and free fatty acids

Fasting plasma concentrations of cholesterol and triglycerides in VLDL, LDL and HDL were determined by a combination of preparative ultracentrifugation, precipitation of apo B-containing lipoproteins and lipid analyses[20]. Triglyceride-rich lipoproteins were subfractionated by cumulative density gradient ultracentrifugation[21]. Consecutive runs calculated to float Sf >400, Sf 60-400 and Sf 20-60 particles were made, and the Sf 12-20 fraction was recovered after the last ultracentrifugal run by slicing the tube 29 mm from the top after the Sf 20-60 lipoproteins had been aspirated. The apo B-48 and apo B-100 concentrations in all fractions were then determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis[22]. LDL peak particle size was determined by subjecting a sample of isolated LDL to high-resolution non-denaturing polyacrylamide gradient (3–7·5%) gel electrophoresis[23]. Plasma free fatty acids were

measured by an enzymatic colorimetric method (Wako Chemicals GmbH, Germany).

Determination of glucose, insulin and proinsulin

Blood glucose was measured by a glucose oxidase method (Kodak Ektachem, Rochester, New York, U.S.A.). Insulin and intact proinsulin were measured by ELISAs based on two monoclonal antibodies (DAKO Insulin and DAKO Intact Proinsulin, DAKO Diagnostics Ltd., Cambridgeshire, U.K.). The monoclonal antibodies used in the insulin assay have very low cross-reactivity with proinsulin. Insulin sensitivity was calculated from the fasting glucose and insulin concentrations using Homeostasis Model Assessment (HOMA)[24].

Carotid artery ultrasound examinations

Measurement of carotid artery intima–media thickness was done according to the European Lacidipine Study on Atherosclerosis ultrasound protocol[25]. The ultrasound device used was a 2000 II s.a., Biosound, Inc., Indianapolis, IN, U.S.A., with an 8 MHz high-resolution annular array scanner. The scannings were recorded on S-VHS videotapes and sent to the Center for Medical Ultrasound, Division of Vascular Ultrasound Research, Wake Forest University, Winston-Salem, NC, U.S.A., for reading. In the present report, only the common carotid artery wall intima–media thickness (mean of right and left artery registrations) is used as a measure of early atherosclerosis, and intima–media thickness henceforth refers to this segment of the carotid artery. The intra-sonographer coefficients of variation were 3·8% and 5·1%, respectively, for the two sonographers. The inter-sonographer coefficient of variation was 4·7%.

Statistical analyses

The individual values of skewed variables were log-normalized before parametric statistical tests. To estimate the overall response of plasma triglycerides during the entire 6-h postprandial period, the total area under the curve or the incremental area under the curve with respect to the fasting plasma triglyceride level were calculated. The associations between TNF-α on the one hand and cellular adhesion molecules and clinical and metabolic variables on the other were assessed by calculation of Spearman rank correlation coefficients. Relationships of TNF-α, cellular adhesion molecules, clinical characteristics and metabolic variables to intima–media thickness were assessed by calculation of univariate Pearson correlation coefficients. A first multivariate model was subsequently generated by multiple stepwise linear regression analysis to identify variables independently correlating with intima–media thickness. Variables showing a significant univariate association with the intima–media thickness variable were included in the multivariate analysis. A second multivariate model was then generated by including TNF-α in the regression equation as a forced variable. In addition, the multivariate models were generated separately in smokers and non-smokers. A forward approach was used for the multivariate analysis, with significance levels set to less than 0·25 to enter and greater than 0·10 to leave the model. All statistical tests were two-sided and *P*-values of less than 0·05 were considered significant.

Table 1 General characteristics of the study group

Number of subjects	96
Smokers (%)	
Current	33
Previous	29
Blood pressure (mmHg)	
Systolic	122 (115–130)
Diastolic	80 (75–85)
BMI ($kg \cdot m^{-2}$)	25·2 (23·7–27·0)
WHR	0·94 (0·90–0·97)
Positive family history of CHD (%)*	12
Common carotid artery IMT (mm)	
Far wall	0·85 (0·75–0·95)
Near wall	0·86 (0·79–0·95)
Cholesterol ($mmol \cdot l^{-1}$)	
Plasma	5·38 (4·63–5·86)
LDL	3·68 (3·09–4·16)
HDL	1·18 (0·93–1·36)
Triglycerides ($mmol \cdot l^{-1}$)	
Plasma	1·08 (0·80–1·68)
Insulin ($pmol \cdot l^{-1}$)	32 (25–49)
Proinsulin ($pmol \cdot l^{-1}$)	2·8 (2·2–3·8)

Values are percentage subjects in group or median (interquartile range). *A positive family history of CHD was considered to be present when coronary heart disease had been diagnosed in at least one first-degree relative under the age of 60. BMI=body mass index; WHR=waist to hip circumference ratio; IMT=intima–media thickness; LDL=low density lipoprotein; HDL=high density lipoprotein.

Table 2 Plasma concentrations of TNF-α and soluble adhesion molecules

TNF-α ($ng \cdot ml^{-1}$)	1·90 (1·60–2·20)
sVCAM-1 ($ng \cdot ml^{-1}$)	322 (293–372)
sICAM-1 ($ng \cdot ml^{-1}$)	170 (148–212)
sE-selectin ($ng \cdot ml^{-1}$)	46 (34–57)

Values are median (interquartile range). TNF-a=tumour necrosis factor-a; sCAM=soluble adhesion molecule; sVCAM-1=soluble vascular cell adhesion molecule-1; sICAM-1=soluble intercellular adhesion molecule-1; sE-selectin=soluble E-selectin.

Results

Basic clinical and metabolic characteristics of the 96 participants are summarized in Table 1 whereas distributions of TNF-α and soluble cellular adhesion molecules are given in Table 2. Two-thirds were current or previous smokers. The vast majority of the participants

Table 3 Relationships of plasma TNF-α concentration to clinical risk indicators and plasma concentrations of soluble cell adhesion molecules

	Tobacco consumption	BMI	WHR	SBP	DBP	sVCAM-1	sICAM-1	sE-selectin
TNF-α	0·105	0·219*	0·144	0·327**	0·333**	0·317**	0·376***	0·350***

Values are Spearmann rank correlation coefficients. BMI=body mass index; WHR=waist-to-hip circumference ratio; SBP=systolic blood pressure; DBP=diastolic blood pressure; sVCAM-1=soluble vascular cell adhesion molecule type-1; sICAM-1=soluble intercellular adhesion molecule type-1; sE-selectin=soluble E-selectin. *P<0·05; **P<0·01; ***P<0·001.

Table 4 Relationships of plasma TNF-α concentration to metabolic risk indicators

	VLDL TG	LDL Chol	HDL Chol	Peak LDL particle size	Insulin	Proinsulin	HOMA IR	FFA	TG AUC
TNF-α	0·306**	0·217*	− 0·290**	− 0·326**	0·188	0·195	0·215*	0·274*	0·358***

Values are Spearmann rank correlation coefficients. VLDL=very low density lipoprotein; LDL=low density lipoprotein; HDL=high density lipoprotein; TG=triglyceride; Chol=cholesterol; HOMA IR=homeostasis model assessment of insulin resistance; FFA=free fatty acids; TG AUC=area under the postprandial triglyceride curve. *P<0·05; **P<0·01; and ***P<0·001.

Table 5 Relationships of plasma TNF-α concentration to plasma concentrations of subfractions of triglyceride-rich lipoproteins in the fasting state and 3 h after intake of a mixed meal

	Sf 60-400 apo B-100		Sf 60-400 apo B-48		Sf 20-60 apo B-100		Sf 20-60 apo B-48	
	0 h	3 h	0 h	3 h	0 h	3 h	0 h	3 h
TNF-α	0·271*	0·308**	0·138	0·198	0·317**	0·220*	0·109	0·132

Values are Spearmann rank correlation coefficients. Sf=Svedberg flotation rate; apo=apolipoprotein *P<0·05; and **P<0·01.

were non-obese, normotensive and without a family history of coronary heart disease. The ultrasound examination revealed that, taken as a group, the 50-year-old men enrolled in the study had a fairly normal common carotid artery intima–media thickness.

Relationships of plasma TNF-α concentration to clinical and metabolic risk indicators for coronary artery disease

To explore whether plasma concentrations of TNF-α are related to levels of established risk indicators for coronary artery disease, univariate Spearman rank correlation coefficients were calculated between TNF-α on the one hand and clinical risk indicators and soluble cellular adhesion molecules (Table 3), major plasma lipoproteins, indices of insulin resistance and postprandial lipaemia (Table 4), and subfractions of triglyceride-rich lipoproteins in the fasting state and during alimentary lipaemia (Table 5) on the other. The plasma TNF-α concentration showed moderately strong positive correlations with the systolic and diastolic blood pressures as well as with the plasma concentrations of all the soluble cellular adhesion molecules examined. Weaker but statistically significant correlations were also found with body mass index. In contrast, the measure of cumulative lifetime tobacco consumption and the waist-to-hip circumference ratio were unrelated to plasma TNF-α. Amongst the metabolic risk indicators, fasting and postprandial VLDLs, both the larger (reflected by the Sf 60-400 apo B-100 concentration) and the smaller particle species (reflected by the Sf 20-60 apo B-100 concentration), correlated positively with TNF-α, whereas chylomicron remnants did not, irrespective of particle size. A fairly strong relation was also found with the degree of alimentary lipaemia after intake of the test meal. In addition, associations were present with the fasting plasma concentrations of LDL cholesterol (positive) and HDL cholesterol (inverse) and the peak LDL particle size, the latter reflecting the LDL subfraction distribution. Furthermore, two of the indices of insulin resistance, the HOMA measure and the postabsorptive free fatty acid concentration, correlated positively with the plasma TNF-α concentration. Basal insulin and proinsulin, on the other hand, were not significantly correlated with TNF-α.

Table 6 Multiple stepwise regression analysis of determinants of common carotid intima–media thickness

Variables considered for inclusion in the multivariate models	Univariate correlation coefficient		
TNF-α	0·253†		
Log TG at 2 h	0·281‡		
Log Sf 60-400 apo B-100 at 3 h	0·243†		
LDL cholesterol	0·261†		
LDL peak particle size	− 0·255‡		
Log basal insulin	0·277†		
Log basal proinsulin	0·278†		
HOMA insulin resistance	0·249†		
sE-selectin	0·299†		
	Regression coefficient	F-to-remove	Increase in multiple R2
Multivariate model I			
sE-selectin	0·002	5·16	0·09
Log TG at 2 h	0·142	6·09	0·05
Multiple R2			0·14
Multivariate model II*			
TNF-α	0·035	4·85	0·06
Log basal insulin	0·126	6·01	0·07
Multiple R2			0·13

TG=triglycerides; Log=log normalized values; Sf=Svedberg flotation rate; Apo=apolipoprotein; HOMA=homeostasis model assessment; sE-selectin=soluble E-selectin. *TNF-α was included as a forced variable. †$P<0·05$; ‡$P<0·01$.

Determinants of common carotid intima–media thickness

The plasma TNF-α concentration, the measured cellular adhesion molecules and all clinical and metabolic risk indicators for coronary artery disease (irrespective of whether they correlated with the plasma TNF-α concentration or not) were evaluated in relation to common carotid intima–media thickness. Table 6 summarizes the variables that correlated significantly with intima–media thickness in univariate analysis and the results of the two multivariate analyses. The plasma TNF-α concentration correlated significantly with common carotid intima–media thickness in univariate analysis. In addition, significant relations with this intima–media thickness measure were seen for the triglyceride concentrations measured in the early postprandial phase, at 1 to 4 h after intake of the test meal, with the strongest correlation attained at 2 h. Of the triglyceride-rich lipoproteins particle measurements, only the large VLDL (Sf 60-400 apo B-100) concentration at 3 h was found to correlate significantly with intima–media thickness. The fasting plasma concentration of the major apo B containing lipoprotein, LDL, was also associated with intima–media thickness. Furthermore, significant correlations existed for basal plasma insulin and proinsulin along with the HOMA index of insulin resistance. Of note, only soluble E-selectin amongst the soluble cellular adhesion molecules determined related to intima–media thickness. Furthermore, none of the clinical risk indicators (blood pressure, cumulative tobacco consumption, alcohol intake, waist-to-hip circumference ratio and body mass index) correlated significantly with intima–media thickness.

In the first multivariate model (Table 6), soluble E-selectin proved to be the strongest determinant of intima–media thickness, accounting for 9% of the variation, whereas the postprandial plasma triglyceride concentration at 2 h after intake of the test meal contributed another 5%. The addition of other variables, including TNF-α, did not significantly increase the value of the multiple R2. In the second model (Table 6), TNF-α was added as a forced variable to the variables considered for inclusion in the first model. Now, basal insulin turned out to be the strongest predictor of intima–media thickness, accounting for 7% of its variation, with TNF-α contributing another 6%. In this model, soluble E-selectin and postprandial triglycerides were no longer significant predictors of intima–media thickness (non-significant *P*-values whereas regression coefficients were largely unchanged).

Separate multivariate analyses in smokers and non-smokers, respectively, showed that postprandial triglycerides and soluble E-selectin were strong determinants of intima–media thickness amongst current smokers (increase in multiple R2 of 0·40 and 0·14, respectively), whereas these variables were not significantly related to

intima–media thickness amongst non-smokers. Of note, in univariate analysis the plasma TNF-α concentration correlated strongly with common carotid intima–media thickness in smokers (r=0·529, $P<0·01$) but not in non-smokers (r=0·155, ns).

Discussion

The present study examined the hypothesis that the proinflammatory cytokine TNF-α influences the atherosclerotic process by causing metabolic perturbations and by increasing the expression of cellular adhesion molecules. On the assumption that plasma TNF-α reflects TNF-α actions in tissues, we investigated the relations of the plasma TNF-α concentration to established clinical and metabolic risk indicators for cardiovascular disease and to plasma levels of soluble cellular adhesion molecules in a representative group of healthy middle-aged men. Common carotid intima–media thickness was used as a surrogate measure of early atherosclerosis. The main finding was that the plasma TNF-α concentration related to a number of established risk indicators, such as systolic and diastolic blood pressure, degree of alimentary lipaemia, plasma VLDL triglyceride and LDL cholesterol concentrations and peak LDL particle size. In addition, two indices of insulin resistance, the HOMA measure and the postabsorptive free fatty acid concentration, correlated positively with the plasma TNF-α concentration. Secondly, the plasma concentrations of all soluble cellular adhesion molecules measured were related to the plasma level of TNF-α. Thirdly, soluble E-selectin and postprandial triglycerides, which were fairly strongly influenced by TNF-α, proved to be independent determinants of common carotid intima–media thickness, taking all other clinical and metabolic risk indicators into account, whereas the plasma TNF-α concentration itself was not. However, in univariate analysis, the plasma TNF-α level correlated significantly with intima–media thickness. In addition, when the effects of TNF-α were adjusted for by including TNF-α as a forced variable in the multivariate analysis, soluble E-selectin and postprandial triglycerides were no longer significantly related to common carotid intima–media thickness. Furthermore, the relationships of soluble E-selectin and postprandial triglycerides to intima–media thickness were largely accounted for by the smokers contained in the study group. It should also be emphasized in this context that the population studied is healthy and contains only a few high-risk men who are smoking, obese and hypertensive, a fact which may have influenced the results. These restrictions notwithstanding, our findings support the notion that increased production of TNF-α is an early event in atherosclerosis and that TNF-α may influence the expression of metabolic and cellular perturbations that are important for the vascular process.

To the best of our knowledge this is the first large-scale clinical study using the carotid intima–media thickness surrogate measure of atherosclerosis that has included plasma TNF-α determinations along with measurements of soluble cellular adhesion molecules and detailed assessments of clinical and metabolic risk indicators. The serum/plasma concentration of TNF-α was studied earlier in postinfarction patients. In a group of young, male postinfarction patients, the plasma concentration of TNF-α turned out to be significantly higher in patients than in age-matched controls[6] and, in a more heterogeneous group, TNF-α was persistently elevated in postinfarction patients at increased risk of recurrent coronary events[26]. The possibility that the elevation of the cytokine in blood in these studies depended on tissue injury caused by the myocardial infarction was addressed by measuring plasma TNF-α at least 3 months after the acute event.

As circulating levels of TNF-α are low and both synthesis and actions of TNF-α are local, the question arises of whether it is relevant to measure the TNF-α concentration in blood. However, a functional C to A polymorphism at position -863 in the promoter of the TNF-α gene is associated with the serum concentration of TNF-α in healthy individuals, suggesting that differences in TNF-α production are reflected in the blood[27].

The positive correlations between the plasma TNF-α concentration and common carotid intima–media thickness and between plasma TNF-α and soluble cellular adhesion molecules in the present study provide human in vivo evidence for the central role of TNF-α expression in atherogenesis. Atherosclerotic lesions start to progress at sites of arterial inflammation (see[28] for review). Dysfunctional endothelial cells become activated and start to express chemokines for monocytes as well as proinflammatory cytokines such as TNF-α. TNF-α then induces the expression of E-selectin, P-selectin, ICAM-1 and VCAM-1 (see[29] for review). The expression of E- and P-selectin on endothelial cells is required for leukocytes to tether and roll on the endothelium. ICAM-1 and VCAM-1 subsequently arrest the leukocytes so they can cross the endothelial layer and enter the underlying tissue. Intimal macrophages derived from monocytes during the next stage start engulfing modified LDL, convert to foam cells and so generate the fatty streak, the precursor of the atherosclerotic plaque. Smaller and denser LDL particles are particularly atherogenic[23], and it is notable in this context that the plasma TNF-α concentration was inversely correlated with LDL peak particle size in the present study.

How then would TNF-α influence lipoprotein metabolism, insulin sensitivity and blood pressure, as indicated by our data? Some information can be gained from reports of experimental studies in animal models and in human subjects. In rodents, infusion of TNF-α leads to an increase in plasma triglyceride and cholesterol concentrations and stimulates hepatic lipid synthesis[30]. TNF-α infusion in mice also produce elevated plasma insulin levels[31], and both the TNF-α mRNA and protein levels are raised in rodent models of obesity and diabetes[4]. Neutralization of TNF-α in one of the

obesity models improved insulin sensitivity, resulting in a significant increase in the insulin-stimulated peripheral uptake of glucose[4]. In addition, expression of TNF-α in adipose tissue is elevated in obese human subjects, and weight reduction decreases the TNF-α mRNA levels in adipose tissue and increases insulin sensitivity[32]. On the molecular level, TNF-α may cause insulin resistance by phosphorylating serine instead of tyrosine in insulin receptor substrate-1[33,34] which then interferes with insulin receptor autophosphorylation and insulin signal transduction. TNF-α can also cause insulin resistance by down-regulating the expression of the insulin responsive glucose transporter GLUT4[35]. Furthermore, TNF-α increases the production of endothelin-1[36] and angiotensinogen[37], which are both implicated in hypertension.

In conclusion, the present study shows that the plasma TNF-α concentration is associated with degree of early atherosclerosis and correlates with metabolic and cellular perturbations that are considered to be important for the atherosclerotic process. It thus supports the notion that increased production of TNF-α is an early and central event in atherogenesis.

This study was supported by grants from the Swedish Medical Research Council (8691, 9533, 12659 and 12660), the Swedish Heart-Lung Foundation, the Marianne and Marcus Wallenberg Foundation, the Petrus and Augusta Hedlund Foundation, the King Gustaf V 80th Birthday foundation and the Foundation for Old Servants.

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