

Relationship of Adiposity to Subclinical Atherosclerosis in Obese Patients with Type 2 Diabetes

Refaat A.F. Hegazi,* Kim Sutton-Tyrrell,† Robert W. Evans,† Lewis H. Kuller,† Steven Belle,† Monica Yamamoto,† Daniel Edmundowicz,* and David E. Kelley*

Abstract

HEGAZI, REFAAT A.F., KIM SUTTON-TYRRELL, ROBERT W. EVANS, LEWIS H. KULLER, STEVEN BELLE, MONICA YAMAMOTO, DANIEL EDMUNDOWICZ, AND DAVID E. KELLEY. Relationship of adiposity to subclinical atherosclerosis in obese patients with type 2 diabetes. *Obes Res.* 2003;11:1597–1605.

Objective: There is an increased prevalence of macrovascular disease in type 2 diabetes. The pathogenesis has been related to metabolic risk factors, insulin resistance, and obesity. One of the strongest predictors is the presence of subclinical atherosclerosis. This study was designed to examine the relationship between obesity and regional patterns of adiposity, insulin resistance, and five independent measures of subclinical atherosclerosis.

Research Methods and Procedures: Fifty-two overweight and obese men and women with type 2 diabetes of relatively short known duration were examined. Measures of subclinical vascular disease were assessment of arterial stiffness by pulse wave velocity, ultrasound measurement of the carotid artery intimal-medial thickness and plaque index, and measurement of the extent of coronary and aortic calcification using electron beam computed tomography. Insulin resistance was measured using the hyperinsulinemic euglycemic clamp. Body composition was measured using DXA and computed tomography.

Results: Adiposity was a strong determinant of pulse wave velocity. Carotid intimal-medial thickness was correlated with age, low-density lipoprotein-cholesterol, and hyperglycemia, but not with adiposity. Hyperglycemia and plasma

activator inhibitor-1 were significant correlates of the carotid artery plaque index. Coronary calcium scores were significantly correlated with age and interleukin-6 and significantly and negatively correlated to insulin sensitivity index.

Discussion: These findings suggest that obesity may play an important role in the early phase of subclinical macrovascular disease related to vessel stiffness, whereas hyperglycemia and insulin resistance in conjunction with other risk factors have important roles in progression from vessel stiffness to atheroma formation in type 2 diabetes.

Key words: regional adiposity, insulin resistance, arterial stiffness, intimal-medial thickness, coronary calcification

Introduction

Cardiovascular disease (CVD)¹ is the leading cause of mortality in type 2 diabetes (1). It is well recognized that one of the strongest predictors of clinically symptomatic vascular disease in type 2 diabetes is the presence of subclinical vascular disease. There are several components to the subclinical manifestations of large vessel pathology. Among these components are arterial stiffness, carotid intimal-medial thickness (IMT), plaque formation, and coronary and aortic calcification. These measures reveal different aspects of vascular disease. For instance, arterial stiffness is a measure of early functional changes of loss of elasticity and compliance within the vascular system (2), whereas IMT and plaque measure a latter phase, one of structural changes involving hyperplasia of arterial smooth

Received for review July 9, 2003.

Accepted in final form October 20, 2003.

*Department of Medicine and †Department of Epidemiology, School of Medicine and Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania.

Address correspondence to David E. Kelley, 809N Montefiore-University Hospital, University of Pittsburgh, 3459 Fifth Avenue, Pittsburgh, PA 15213.

E-mail: kelley@msx.dept-med.pitt.edu

Copyright © 2003 NAASO

¹ Nonstandard abbreviations: CVD, cardiovascular disease; IMT, intimal-medial thickness; PWV, pulse wave velocity; FFM, fat-free mass; CT, computed tomography; AT, adipose tissue; HU, Hounsfield unit(s); HbA_{1c}, hemoglobin A_{1c}; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; ISI, insulin sensitivity index; CCS, coronary calcium score; ACS, aortic calcium score; PAI-1, plasminogen activator inhibitor-1; IL, interleukin; TNF- α , tumor necrosis factor- α ; CRP, C-reactive protein.

muscle and the subintimal deposition of cholesterol plaques (3). Coronary calcium represents a relatively advanced phase of atherosclerotic plaques (4).

Arterial stiffness can be measured noninvasively by determinations of pulse wave velocity (PWV) and has been shown to predict risk of future CVD events (5) and correlate with reduced blood volume delivery to the lower extremities (6). The underlying basis of arterial stiffness seems to be compromised function of elastin within the vessel wall, which, at least in early stages, is potentially reversible (7). Similarly, the assessment of IMT by ultrasound examination of the carotid artery predicts future coronary events. Hodis et al. (3) have shown that for each 0.13-mm increment in common carotid arterial IMT, the risk increased 1.4 times for myocardial infarction, coronary death, or any coronary event. Coronary calcification is considered a sign of complicated atherosclerotic plaques. The degree of coronary artery calcification is strongly related to coronary atherosclerosis as measured at postmortem examination, by coronary angiography, or by coronary ultrasound (8–10). Similarly, calcifications of the aorta represent an advanced stage of intimal atherosclerosis. In the Framingham cohort, calcification in the thoracic and abdominal aorta has been associated with an increased risk of cardiovascular disease in men and women (11).

The pathogenesis of macrovascular disease has been attributed to a number of metabolic risk factors associated with type 2 diabetes, including hyperglycemia, hypertension, dyslipidemia, obesity, insulin resistance, and various other metabolic perturbations associated with the metabolic syndrome such as pro-inflammatory cytokines (12). Our understanding of how these risk factors interact and coalesce to cause clinical manifestations of macrovascular disease is incomplete. In patients with type 2 diabetes who have clinically symptomatic macrovascular disease, it can be very difficult to unravel the respective contributions of various risk factors because these typically co-exist. A potentially useful approach is to examine the association of risk factors at earlier stages of macrovascular disease with subclinical vascular disease. In this study, assessments of PWV, carotid IMT, and coronary and aortic calcification were obtained in a cohort of overweight and obese research volunteers with type 2 diabetes of relatively short known duration, who did not have any clinical manifestations of large vessel pathology.

Research Methods and Procedures

Research Volunteers

Fifty-two research volunteers were recruited from the general community in response to public advertisements. Inclusion criteria were a prior diagnosis of type 2 diabetes of known duration not longer than 5 years, age between 20 and 70 years, and stable weight (defined as current weight

± 3 kg during the past 6 months), with overall good general health other than type 2 diabetes. Those individuals treated with insulin were excluded, as were potential volunteers with a prior history of myocardial infarction, stroke, or peripheral vascular disease. Because smoking may affect markers of inflammation (i.e., cytokines), those with current use of tobacco were excluded. All volunteers had a medical examination at screening with standard laboratory testing, and only those with normal values for liver and renal function were included. The project was approved by the University of Pittsburgh Institutional Review Board, and all volunteers provided written informed consent before participation.

Measurement of Insulin Sensitivity and Body Composition

Insulin sensitivity was measured using the hyperinsulinemic euglycemic glucose clamp technique, as previously described (13). On the preceding day, subjects were admitted to the University of Pittsburgh General Clinical Research Center, and after an overnight fast, in the morning, a catheter was placed in a forearm vein for infusion of glucose and insulin. An additional intravenous catheter was inserted in a retrograde direction on the dorsum of the opposite hand for blood sampling, and the hand was heated to arterialize blood. Fasting samples were collected for determination of serum insulin, glucose, fatty acids, and lipoproteins. A continuous infusion of regular insulin (Humulin; Eli Lilly, Indianapolis, IN) was given at a rate of 40 mU/m² per minute for 4 hours, and plasma glucose was measured using a glucose/lactate analyzer (YSI-2300 Plus; Yellow Springs Instruments, Yellow Springs, OH) at 5-minute intervals during the clamp. The target blood glucose level was 90 mg/dL, and in this cohort with type 2 diabetes with fasting hyperglycemia, plasma glucose was initially permitted to decline until it reached euglycemia, and then it was maintained using an adjustable infusion of 20% dextrose. Systemic fat mass and fat-free mass (FFM) were assessed by DXA, as previously described (13). Computed tomography (CT) was used to assess regional distribution of adipose tissue (AT) in the abdomen and thigh and fat content within skeletal muscle and liver, as previously described in detail. CT was used to evaluate hepatic fat content, and the average value in Hounsfield unit(s) (HU) for four regions of interest was used for liver attenuation. These values were normalized to the attenuation value of the spleen to calculate the liver-to-spleen ratio as a parameter of relative fat content in the liver. A liver-to-spleen ratio ≤ 1.0 is indicative of a fatty liver.

Measurement of Subclinical Atherosclerosis

PWV: The PWV was measured at the Ultrasound Research Laboratory at the University of Pittsburgh Graduate School of Public Health. Simultaneous Doppler flow signals

were obtained from the right carotid and right femoral arteries using nondirectional transcutaneous Doppler flow probes (model 810A, 9.0- to 10-MHz probes; Parks Medical Electronics, Inc., Aloha, OR). Digitized data were recorded by custom programming for subsequent analysis. At least 10 beats were averaged for each simultaneous recording site using the QRS complex for synchronization. Three separate runs were recorded for each participant, and all usable runs were averaged. PWV was calculated as the quotient of the distance between the carotid and femoral arteries divided by the time interval between carotid and femoral waveforms. Stiffer vessels are associated with a faster PWV.

IMT and Detection of Focal Plaque: A Toshiba SSA-270A scanner equipped with a 5-MHz linear array imaging probe was used to scan the right and left common carotid artery, the carotid bulb, and the first 1.5 cm of the internal and external carotid arteries. For each location, the vessel was imaged in multiple planes, focusing on the interfaces required to measure IMT and on any areas of focal plaque. Images were taped and later digitized for scoring. Trained readers measured the average IMT across 1-cm segments of the near and far walls of the distal common carotid artery and the far wall of the carotid bulb and the internal carotid artery on both right and left sides. Measures from each location were averaged to produce an overall measure of IMT. A computerized reading program developed for the Cardiovascular Health Study and modified in Pittsburgh was used. Readers also scored the ultrasound images for plaque in the proximal common artery, distal common artery, carotid bulb, internal carotid artery, and external carotid artery. Plaque was defined as a distinct area of hyperechogenicity and/or protrusion into the lumen of the vessel with at least 50% greater thickness than the surrounding area. For each segment, the degree of plaque was graded as follows: 0 = no plaque; 1 = 1 small plaque, <30% of vessel diameter; 2 = 1 medium plaque between 30% and 50% of the vessel diameter or multiple small plaques; and 3 = 1 large plaque >50% of the vessel diameter or multiple plaques with at least 1 medium plaque. The grades were summed across the right and left carotid arteries to create an overall measure of the extent of focal plaque (14).

Electron Beam Computed Tomography: For the detection of coronary artery calcium, consecutive single-slice, 100-ms scans were performed. During scanning, epicardial coronary artery motion was "frozen"; thus, blurring of vessel borders caused by "motion-unsharpness" artifact was minimal. In-plane spatial resolution was ~0.6 mm, and imaged opacities (densities) were accurately localized in three-dimensional space. The entire procedure lasts ~10 minutes, requires minimal patient effort (only breath holding), and requires no contrast. Coronary calcium score was considered present when a density of >130 HU occurred over an area $\geq 1 \text{ mm}^2$. The calcium score was calculated from the product of the scaling factor and the area of calcification (millimeters

squared). HU were detected in four contiguous pixels overlying the coronary arteries. The following scaling factors were assigned to ranges of densities: 130 to 199 HU = 1; 200 to 299 HU = 2; 300 to 399 HU = 3; ≥ 400 HU = 4 (15). The calcium scores from lesions within each major epicardial artery were summed to obtain the score for each artery. Because the distinction between the distal left main and the proximal left anterior descending arteries was often unclear, calcium in the region of these arteries was considered as one region, referred to as left main/left anterior descending artery. The total calcium score for a patient was calculated as the sum of calcium scores from each epicardial artery.

Statistical Analysis

Neither of the continuous variables (PWV and IMT) was normally distributed. Univariate associations between the continuous measures were performed using Spearman correlations. The plaque index was divided into two groups: those without plaque (low risk: plaque index = 0) and those with plaque (high risk: plaque index = 1+). In addition, and because the coronary and aortic calcium are testing discrete areas of the subclinical disease, these measures were divided at the 75th percentile into high- and low-risk groups. The association between this dichotomous measure and other variables was assessed using *t* tests and Wilcoxon tests as appropriate for continuous variables. A linear regression model was employed for log-transformed PWV and IMT and a logistic regression model for dichotomous variables. All analyses were performed using the Statistical Packages for Social Sciences (SPSS, version 11, SPSS, Chicago, IL). For statistical analysis, testing was two-sided, and $p < 0.05$ was considered statistically significant.

Results

Clinical Characteristics and Body Composition

Shown in Table 1 are the clinical characteristics of the 52 study participants with type 2 diabetes. A majority of the research volunteers were women (65%), and the group was middle-aged, with a mean age of 51 years. All the volunteers were either overweight or obese, with a range in BMI of 27.0 to 49.4 kg/m². The baseline hemoglobin A_{1c} (HbA_{1c}) was $8 \pm 1.6\%$, with a range of 5% to 13%. Lipid profiles are also shown in Table 1. Although the group mean values were consistent with the presence of a dyslipidemia, characteristic of type 2 diabetes, just two of the volunteers had a lipid profile in a high-risk category of high-density lipoprotein-cholesterol (HDL-C) <40 mg/dL, low-density lipoprotein-cholesterol (LDL-C) >160 mg/dL, and total cholesterol >240 mg/dL, as based on consensus criteria (National Cholesterol Education Program). The mean value for the insulin sensitivity index (ISI), measured by glucose clamp, was indicative of the insulin resistance that is char-

Table 1. Characteristics of recently diagnosed type 2 diabetic patients ($n = 52$)

Parameter	Mean (SD)	Minimum to maximum
Age (years)	51 (9)	27 to 67
BMI (kg/m ²)	35 (5)	27 to 49
Systolic blood pressure (mm Hg)	138 (17)	104 to 182
Diastolic blood pressure (mm Hg)	80 (8)	64 to 100
Plasma glucose (mg/dL)	166 (54)	90 to 298
HbA _{1c} (%)	8 (2)	5 to 13
Cholesterol (mg/dL)	210 (33)	135 to 288
Triglycerides (mg/dL)	168 (150)	80 to 885
HDL (mg/dL)	48 (21)	27 to 66
LDL (mg/dL)	123 (32)	72 to 201
VLDL-C (mg/dL)	28 (10)	13 to 55
Serum insulin (uU/mL)	16.9 (11.1)	4.2 to 70.8
ISI (mg/min/kg FFM)	2.3 (2.1)	0 to 11.7
Visceral fat (cm ²)	252 (90)	27 to 49
Abdominal subcutaneous fat (cm ²)	433 (117)	178 to 685
Thigh fat (cm ²)	308 (121)	102 to 587
Hepatic fat (HU)	46 (10)	23 to 62
CRP (μ g)	3.4 (2.6)	0.2 to 10.3
TNF- α	3.9 (1.1)	1.7 to 7.6
IL-6	2.5 (1.4)	0.6 to 7.7
PAI-1	78.5 (47.2)	28.9 to 218.8
Fibrinogen	248.8 (82.9)	82 to 493

VLDL-C, very-low-density lipoprotein-cholesterol.

acteristic of type 2 diabetes. Values for regional distribution of AT are also shown in Table 1.

Measures of Subclinical Atherosclerosis

The mean values for PWV (available for 50 volunteers) and for carotid artery IMT (available for 45 persons) are shown in Figure 1. Values for PWV ranged from 400 to 1400 cm/s, with a mean value of 820 cm/s. Mean IMT was 79 mm. The prevalence of any focal plaque was 43%. The median plaque index was 0 (maximum is 3); 8% of the study persons had an index of 2, and 13% had an index of 3. Coronary calcium scores for 48 subjects were available. The mean score was 43, and the median score was 12. Aortic calcium scores were available for 48 subjects; 14% had a score of <10, and 56% had a score of <100. The mean was 490, and the median score was 59.

Correlates of Measures of Subclinical Atherosclerosis

Table 2 shows the Spearman correlation of the risk factors with PWV and IMT. Because of prior reports indicating a consistent association between both age and systolic blood pressure and PWV (16), partial Spearman correlation of PWV with cardiovascular risk factors, adjusted for age and systolic blood pressure, are also shown in Table 2. Of particular note, each measure of adiposity was correlated with PWV, and of those, BMI had the strongest simple correlation. Also shown in Table 2 are the patterns of association for carotid artery IMT. These patterns differed from those observed for PWV. There was a significant and positive correlation of IMT with age, fasting hyperglycemia, HbA_{1c}, and LDL-C. However, unlike the pattern observed for PWV, none of the measures of adiposity correlated significantly with IMT. Interestingly, insulin sensitivity and fasting hyperinsulinemia were not significantly correlated with either PWV or IMT in this cohort of obese type 2 diabetic patients and neither were markers of inflammation and thrombosis (data not shown).

The distribution of potential risk factors for either low or high values of plaque index, coronary calcium score (CCS), and aortic calcium score (ACS) are shown in Table 3. With respect to the plaque index, HbA_{1c}, in addition to plasminogen activator inhibitor-1 (PAI-1), was significantly higher in those with higher plaque scores, but insulin sensitivity and adiposity were not significantly different. Age, serum insulin, and interleukin (IL)-6 were significantly higher among those subjects with higher CCS, whereas insulin sensitivity was significantly lower. In this group with higher ACS, age, systolic blood pressure, and LDL-C were significantly higher. Other markers of inflammation or thrombosis [tumor necrosis factor- α (TNF- α), C-reactive protein (CRP), PAI-1, and fibrinogen] did not differ with respect to CCS or ACS.

Multivariate Correlations for Measures of Subclinical Atherosclerosis

Finally, to examine for potential interaction among risk factors with differing steps of subclinical vascular disease, multivariate regression analysis was performed (Table 4). With this approach, BMI, hepatic fat content, and diastolic blood pressure were found to be independently and significantly associated with PWV, and this model explained 49% of the variability in PWV. In regard to carotid IMT, age and glucose were significantly and independently associated with IMT, explaining 33% of the variability for values of IMT. The only independently related risk factor with plaque index was HbA_{1c}. Age and ISI were the significant independent predictors of CCS, explaining 37% of the variability of CCS. Age was the only risk factor independently associated with aortic calcifications.

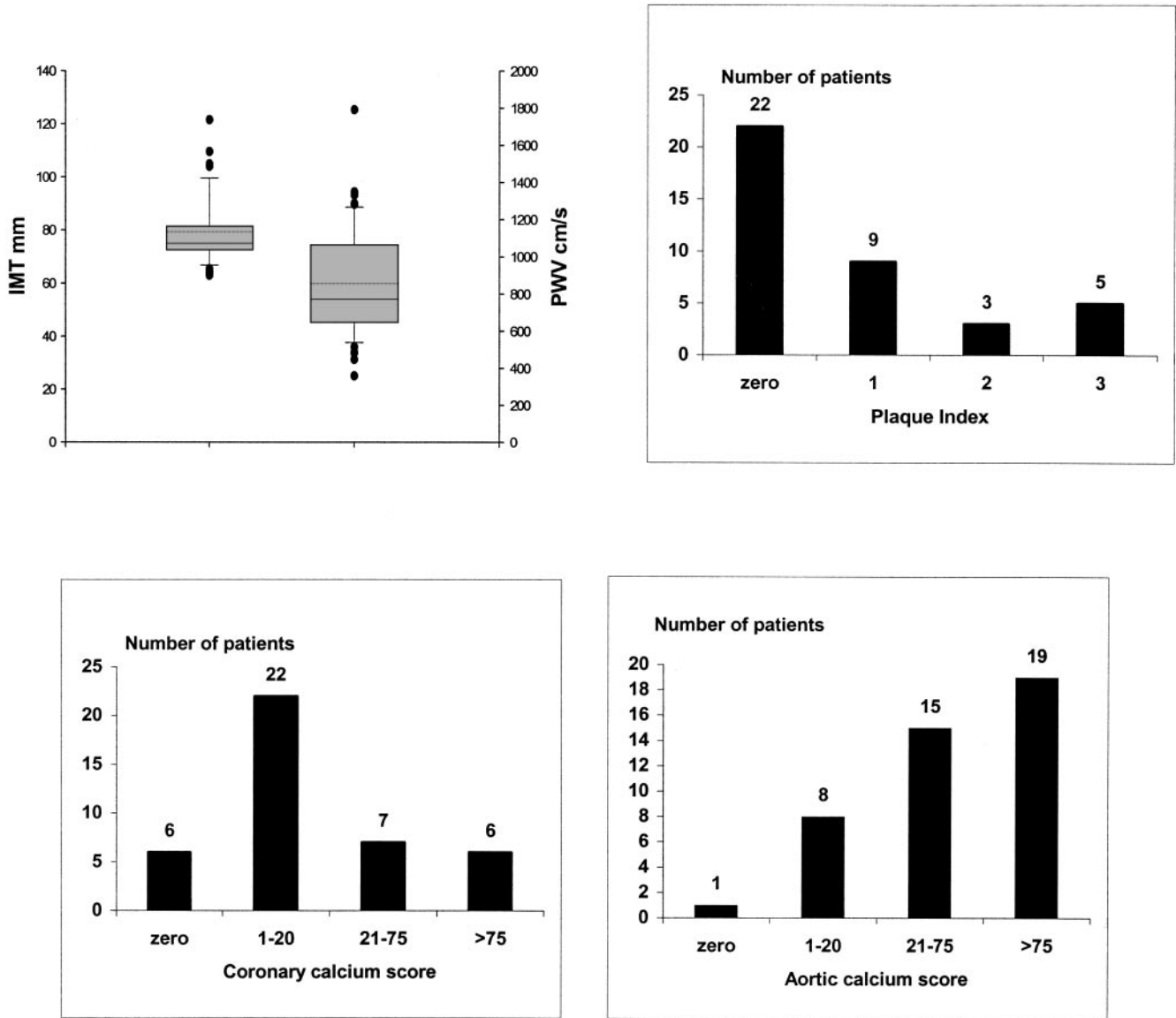


Figure 1: Distribution of five measures of subclinical atherosclerosis.

Discussion

In this study, different aspects of the subclinical manifestations of CVD were assessed in a cohort of individuals with type 2 diabetes and examined for potential relationship to a panel of CVD risk factors, including detailed assessments of insulin sensitivity and body composition. A strong association of PWV with various measures of adiposity was observed. On the contrary, IMT and coronary calcification were independently correlated with hyperglycemia and insulin resistance, respectively. Thus, the key findings in this study are that obesity acts as a determinant of the early functional changes of the arterial tree as measured by the arterial stiffness, whereas hyperglycemia and insulin resis-

tance act as determinants of the later structural and atherotic changes as measured by the carotid IMT and coronary calcification.

In this study, many of the measures of adiposity were related to arterial stiffness. These findings are in agreement with previous reports of the positive association between obesity and PWV (17,18). There are a number of potential mechanisms by which obesity might affect arterial stiffness. Apart from induction of insulin resistance (19), increased influx of free fatty acids has been proposed as a possible explanation for the association between central obesity and PWV (20). Free fatty acids may contribute to vascular stiffness by increasing adrenergic reactivity, vascular tone,

Table 2. Spearman correlation of the risk factors and PWV and IMT

Variable	Spearman correlation with PWV		Age and systolic blood pressure adjusted Spearman correlation with PWV		Spearman correlation with IMT	
	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>
Age (years)	0.18	0.21			0.49	0.00
BMI (kg/m ²)	0.58	0.001	0.59	0.001	0.10	0.52
Systolic blood pressure (mm Hg)	0.13	0.37			0.25	0.10
Diastolic blood pressure (mm Hg)	0.28	0.04	0.20	0.19	0.22	0.15
Plasma glucose (mg/dL)	0.08	0.58	0.05	0.77	0.38	0.01
HbA _{1c} (%)	0.08	0.60	0.06	0.72	0.29	0.04
Total cholesterol (mg/dL)	-0.04	0.76	-0.09	0.59	0.28	0.07
LDL-C (mg/dL)	-0.04	0.78	-0.12	0.45	0.34	0.02
Serum insulin (μ U/ml)	0.15	0.29	0.13	0.42	0.15	0.33
ISI (mg/min/kg FFM)	-0.08	0.57	0.20	0.19	0.15	0.35
Plasma free fatty acids (μ M)	0.23	0.13	0.21	0.18	-0.07	0.70
Visceral fat (cm ²)	0.23	0.08	0.29	0.06	0.11	0.46
Abdominal subcutaneous fat (cm ²)	0.43	0.003	0.17	0.29	0.06	0.73
Thigh fat (cm ²)	0.38	0.007	0.31	0.046	0.11	0.48
Hepatic fat (HU)	0.47	0.001	0.43	0.005	0.08	0.62

and blood pressure (21). Although, in this study, the relation of free fatty acids to PWV was not statistically significant, perhaps because of the small sample size, PWV was independently related to hepatic fat content. Fatty liver is considered to be a part of the insulin resistance syndrome and is common in type 2 diabetics (22). Recently, we have shown that hepatic fat is strongly related to a number of cardiovascular risk factors (insulin resistance, dyslipidemia, and inflammatory markers) in type 2 diabetic patients (23). To our knowledge, this relation of fatty liver to PWV is a novel observation and one that merits further research.

In the pathogenesis of atherosclerosis, IMT is regarded as a later development than arterial stiffness. In this cohort of research volunteers with type 2 diabetes, carotid artery IMT was correlated with hyperglycemia but not with obesity or insulin resistance. Whether this statistical association is a manifestation of a biological cause-and-effect relationship remains to be elucidated. In the analysis of the entire cohort of individuals with type 2 diabetes followed in the United Kingdom Prospective Diabetes Study, there was a significant relationship between glycemic control and macrovascular complications (24). Moreover, there is evidence that elevated glucose levels can, through glycation, play a role in increasing the amounts of oxidized LDL-C, which is capable of injuring the endothelial layer and can be deposited in the subintimal space, leading to the formation of cholesterol plaques (25,26).

Whereas IMT measures the thickness of the carotid arterial walls (comprising the intimal and the medial layers), the plaque index measures the extent of the subintimal atherosclerotic plaques deposited in the walls of the carotid arteries. Thus, plaque index is considered to be a measure of a later phase of atherosclerosis. In addition to HbA_{1c}, the carotid plaque index related positively with PAI-1. PAI-1 is highly expressed in AT from obese subjects (27). It increased neointima formation in balloon-injured rat carotid arteries (26) and is thought to be a risk marker of atherosclerosis and type 2 diabetes (28).

Coronary calcium is considered as an index of the total atherosclerosis burden and has been found to be a strong predictor of diabetes-specific cardiovascular complications in both type 1 (29) and type 2 (30) diabetes. In this study, age and the ISI were independently and significantly related to the degree of coronary calcifications. These findings are in agreement with the observations that insulin resistance (measured by the homeostasis model assessment) is positively associated with coronary atherosclerosis (31). This consistent association between insulin resistance and coronary calcifications may be explained by the effects of the cluster of metabolic changes associated with insulin resistance (hypertension, hyperglycemia, dyslipidemia, and hyperinsulinemia; the insulin resistance syndrome). However, here, only serum insulin was a positive correlate of the degree of CCS, which agrees with previous studies (31).

Table 3. Associations between risk factors and plaque index, coronary calcium, and aortic calcium

Variable	Plaque			CCS			ACS		
	Mean value	Mean value	p	Mean value	Mean value	p	Mean value	Mean value	p
	No Plaque (n = 25)	Plaque (n = 20)		CCS < 50 (n = 34)	CCS > 50 (n = 10)		ACS < 593 (n = 31)	ACS > 593 (n = 11)	
Age (years)	48	53	0.09	50	56	0.03	49	57	0.005
BMI (kg/m ²)	34	36	0.19	35	38	0.054	35	36	0.71
Systolic blood pressure (mm Hg)	134	142	0.06	138	143	0.38	136	148	0.035
Diastolic blood pressure (mm Hg)	80	82	0.33	81	79	0.43	81	81	0.95
Plasma glucose (mg/dL)	162	184	0.13	176	189	0.52	177	184	0.71
HbA _{1c} (%)	7.6	8.7	0.02	8.3	8.6	0.61	8.3	8.5	0.76
Total cholesterol (mg/dL)	200	204	0.70	202	219	0.18	200	222	0.07
LDL-C (mg/dL)	123	129	0.53	125	141	0.19	122	145	0.046
Serum insulin (μU/ml)	15	19	0.29	15	23	0.04	16	18	0.58
ISI (mg/min/kg FFM)	2.4	2.2	0.77	2.5	1.4	0.04	2.0	3.2	0.09
CRP (μg)	3.3	3.5	0.53	3.8	3.5	0.75	3.6	4.3	0.43
TNF-α	3.9	3.6	0.61	3.8	4.1	0.60	3.7	4.4	0.08
PAI-1	71	98	0.02	77.8	84.0	0.71	76.4	87.2	0.54
IL-6	2.4	2.5	0.63	2.2	3.9	0.001	2.3	3.2	0.09
Fibrinogen	257	261	0.94	247.4	242.7	0.89	248.4	240.7	0.80

Increased insulin stimulates the sympathetic nervous system, resulting in increased heart rate and blood pressure. In addition, insulin may cause hypertrophy of the vascular wall, resulting in an increase in the number and size of monocytes, increases in collagen, and proliferation of smooth muscle cells (17).

In this study, the presence of aortic calcification was strongly and independently related to age. Consistent with the findings of this study, it has been previously noted that the presence of aortic calcification is strongly related to age (11). Similarly, our finding that aortic calcium scores were significantly higher than coronary calcium scores is consis-

Table 4. Independent associations of the risk factors with measures of subclinical disease

	PWV			IMT			Plaque*		CCS*		ACS*	
	B	Std. B	p	B	Std. B	p	B	p	B	p	B	p
Age				0.67	0.47	0.001			0.39	0.02	0.34	0.04
BMI	0.27	0.45	0.001									
Diastolic blood pressure	0.12	0.30	0.01									
Glucose				0.25	0.25	0.05						
Hb _{A1c}							0.39	0.03				
ISI									-0.46	0.02		
Hepatic fat	0.11	0.33	0.01									
PAI-1							0.10	0.09				
Model R ²	0.49			0.33			0.12		0.37		0.26	

* Logistic Regression.

tent with a study by Kuller et al., suggesting that aortic calcification may develop before coronary calcification (32).

This study is the first report of the relationships among regional body adiposity, insulin resistance, and five measures of subclinical atherosclerosis. A few methodological issues should be addressed in the current investigation. There is a possibility of type 2 or type 1 errors due to the relatively small sample size, which is, in part, caused by the time-consuming and expensive nature of the euglycemic clamp studies. However, the reliability and accuracy of the clamp procedure for assessment of insulin resistance, as well as the CT measures of body fat distribution, help reduce imprecision in these assessments. Moreover, the significant associations of the risk factors and measures of subclinical disease suggest the strength of the power of these associations at the early phases of the macrovascular disease in type 2 diabetes. Finally, there were strong inter-correlations among the measures of subclinical vascular disease. Interestingly, in regression analyses, arterial stiffness and IMT were associated with the degree of coronary atherosclerosis independently of metabolic risk factors (data not shown), suggesting that PWV and IMT can be used as noninvasive surrogates for assessing the risk of macrovascular disease at early phases of type 2 diabetes.

In conclusion, age is a major correlate of subclinical atherosclerosis. In addition, obesity indices, and especially BMI and hepatic fat, independently correlated with arterial stiffness as measured by PWV in obese type 2 diabetic patients, whereas the later arterial structural abnormalities (IMT and the coronary calcification) were independently associated with hyperglycemia and insulin resistance, respectively. Complicated atherosclerosis, as estimated by the extent of coronary calcification, was independently related to age and insulin resistance. These findings may help outline the chronological order of the pathological changes within the arterial system resulting in macrovascular disease associated with type 2 diabetes. It can also be suggested that, in the early stages of type 2 diabetes, weight reduction could modify cardiovascular risk by improving glycemic control, reversing arterial stiffness, and reducing blood pressure.

Acknowledgments

This work was supported by a research grant from Hoffman-La Roche, Nutley, NJ; the University of Pittsburgh Obesity and Nutrition Research Center (P30, DK 046,204); and the General Clinical Research Center (MO1 RR00056). We thank Therese McKolanis and Cynthia Kern for technical assistance.

References

1. **Haffner SM, Lehto S, Ronnema T, Pyorala K, Laakso M.** Mortality from coronary heart disease in subjects with type 2

- diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med.* 1998;339:229–34.
2. **Arnett DK, Evans GW, Riley WA.** Arterial stiffness: a new cardiovascular risk factor? *Am J Epidemiol.* 1994;140:669–82.
3. **Hodis HN, Mack WJ, LaBree L, et al.** The role of carotid arterial intima-media thickness in predicting clinical coronary events. *Ann Intern Med.* 1998;128:262–9.
4. **Rumberger JA, Simons DB, Fitzpatrick LA, Sheedy PF, Schwartz RS.** Coronary artery calcium area by electron-beam computed tomography and coronary atherosclerotic plaque area. A histopathologic correlative study. *Circulation.* 1995;92:2157–62.
5. **Lehmann ED, Hopkins KD, Gosling RG.** Increased aortic stiffness in women with NIDDM. *Diabetologia.* 1996;39:870–1.
6. **Suzuki E, Kashiwagi A, Nishio Y, et al.** Increased arterial wall stiffness limits flow volume in the lower extremities in type 2 diabetic patients. *Diabetes Care.* 2001;24:2107–14.
7. **O'Rourke M.** Mechanical principles in arterial disease. *Hypertension.* 1995;26:2–9.
8. **Baumgart D, Schmermund A, Goerge G, et al.** Comparison of electron beam computed tomography with intracoronary ultrasound and coronary angiography for detection of coronary atherosclerosis. *J Am Coll Cardiol.* 1997;30:57–64.
9. **Fiorino AS.** Electron-beam computed tomography, coronary artery calcium, and evaluation of patients with coronary artery disease. *Ann Intern Med.* 1998;128:839–47.
10. **Thompson GR, Forbat S, Underwood R.** Electron-beam CT scanning for detection of coronary calcification and prediction of coronary heart disease. *QJM.* 1996;89:565–70.
11. **Wittman JC, Kannel WB, Wolf PA, et al.** Aortic calcified plaques and cardiovascular disease (the Framingham Study). *Am J Cardiol.* 1990;66:1060–4.
12. **Geiss LS, Smith PJ, National Diabetes Data Group.** *Diabetes in America.* Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases; 1995, pp. 233–57.
13. **Kelley DE, Goodpaster B, Wing RR, Simoneau JA.** Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *Am J Physiol.* 1999;277:E1130–41.
14. **Sutton-Tyrrell K, Lassila HC, Meilahn E, Bunker C, Matthews KA, Kuller LH.** Carotid atherosclerosis in premenopausal and postmenopausal women and its association with risk factors measured after menopause. *Stroke.* 1998;29:1116–21.
15. **Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R.** Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol.* 1990;15:827–32.
16. **Asmar R, Benetos A, London G, et al.** Aortic distensibility in normotensive, untreated and treated hypertensive patients. *Blood Press.* 1995;4:48–54.
17. **Sutton-Tyrrell K, Newman A, Simonsick EM, et al.** Aortic stiffness is associated with visceral adiposity in older adults enrolled in the study of health, aging, and body composition. *Hypertension.* 2001;38:429–33.

18. **Mackey RH, Sutton-Tyrrell K, Vaitkevicius PV, et al.** Correlates of aortic stiffness in elderly individuals: a subgroup of the Cardiovascular Health Study. *Am J Hypertens.* 2002;15:16–23.
19. **Björntorp P.** Metabolic implications of body fat distribution. *Diabetes Care.* 1991;14:1132–43.
20. **Longo R, Ricci C Masutti F, et al.** Fatty infiltration of the liver. Quantification by 1H localized magnetic resonance spectroscopy and comparison with computed tomography. *Invest Radiol.* 1993;28:297–302.
21. **Egan BM, Lu G, Greene EL.** Vascular effects of non-esterified fatty acids: implications for the cardiovascular risk factor cluster. *Prostaglandins Leukot Essent Fatty Acids.* 1999;60:411–20.
22. **James O, Day C.** Non-alcoholic steatohepatitis: another disease of affluence. *Lancet.* 1999;353:1634–6.
23. **Kelley DE, McKolanis TM, Hegazi RA, Kuller LH, Kalhan SC.** Fatty liver in type 2 diabetes mellitus: relation to regional adiposity, fatty acids, and insulin resistance. *Am J Physiol.* 2003;285:E906–16.
24. **Group UKPDS.** UK Prospective Diabetes Study 6. Complications in newly diagnosed type 2 diabetic patients and their association with different clinical and biochemical risk factors. *Diabetes Res.* 1990;13:1–11.
25. **Lyons TJ.** Glycation and oxidation: a role in the pathogenesis of atherosclerosis. *Am J Cardiol.* 1993;71:26B–31B.
26. **DeYoung MB, Tom C, Dichek DA.** Plasminogen activator inhibitor type 1 increases neointima formation in balloon-injured rat carotid arteries. *Circulation.* 2001;104:1972–81.
27. **Bastelica D, Mavri A, Verdierl M, Berthet B, Juhan-Vague I, Alessi MC.** Relationships between fibrinolytic and inflammatory parameters in human adipose tissue: strong contribution of TNFalpha receptors to PAI-1 levels. *Thromb Haemost.* 2002;88:481–7.
28. **Festa A, D'Agostino R Jr, Williams K, et al.** The relation of body fat mass and distribution to markers of chronic inflammation. *Int J Obes Relat Metab Disord.* 2001;25:1407–15.
29. **Olson JC, Edmundowicz D, Becker DJ, Kuller LH, Orchard TJ.** Coronary calcium in adults with type 1 diabetes: a stronger correlate of clinical coronary artery disease in men than in women. *Diabetes.* 2000;49:1571–8.
30. **Yoshida M, Takamatsu J, Yoshida S, et al.** Scores of coronary calcification determined by electron beam computed tomography are closely related to the extent of diabetes-specific complications. *Horm Metab Res.* 1999;31:558–63.
31. **Arad Y, Newstein D, Cadet F, Roth M, Guerci AD.** Association of multiple risk factors and insulin resistance with increased prevalence of asymptomatic coronary artery disease by an electron-beam computed tomographic study. *Arterioscler Thromb Vasc Biol.* 2001;21:2051–8.
32. **Kuller LH, Matthews KA, Sutton-Tyrrell K, Edmundowicz D, Bunker CH.** Coronary and aortic calcification among women 8 years after menopause and their premenopausal risk factors: the Healthy Women Study. *Arterioscler Thromb Vasc Biol.* 1999;19:2189–98.