0021-972X/05/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 90(3):1495–1501
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doi: 10.1210/jc.2004-1579

The Association between Abdominal Visceral Fat and Carotid Stiffness Is Mediated by Circulating Inflammatory Markers in Uncomplicated Type 2 Diabetes

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Central obesity, insulin resistance, inflammation, as well as vascular changes are common in patients with type 2 diabetes. In this study we assessed the relationship among stiffness of the carotid artery, visceral fat, and circulating inflammatory markers in type 2 diabetic subjects. Carotid stiffness, quantified as the distensibility coefficient (DC), was measured by ultrasound in asymptomatic, normotensive patients with uncomplicated, well-controlled type 2 diabetes and in controls. Body fat distribution was quantified by magnetic resonance imaging. In patients, the carotid DC was inversely associated with visceral fat area ($r = -0.660$; $P = 0.005$) and plasma levels of C-reactive protein (CRP; $r = -0.687$; $P = 0.002$), but most strongly with plasma IL-6 ($r = -0.766$; $P < 0.001$). In multivariate analysis, the association between DC and visceral fat disappeared after adjustment for CRP and IL-6. Correction for age, body mass index, blood pressure, glycosylated hemoglobin, or fasting plasma glucose did not affect the association between carotid DC and inflammatory markers. Thus, carotid stiffness is associated with visceral obesity in patients with uncomplicated type 2 diabetes, but this association seems to be mediated by circulating IL-6 and CRP, of which IL-6, at least in part, originates from adipose tissue and stimulates hepatic CRP production. (*J Clin Endocrinol Metab* 90: 1495–1501, 2005)

NUMEROUS STUDIES HAVE shown that arterial stiffness is a strong predictor of cardiovascular disease (CVD) risk (1–3). Arterial stiffness increases with aging, but premature arterial stiffening occurs in subjects at high risk of CVD, including patients with hypertension, end-stage renal disease, coronary heart disease, and diabetes mellitus (1–4). Large artery stiffening involves structural changes, including fragmentation and degeneration of elastin, increases in collagen, and thickening of the vessel wall (4), but recent studies also suggest that endothelial dysfunction may play a pathophysiological role (5). Arterial stiffening seems to predispose for atherosclerotic plaque formation (5). Components of the metabolic syndrome, such as visceral obesity, insulin resistance, hyperglycemia, hypertriglyceridemia, and hypertension may promote arterial stiffening (6–8). Insulin resistance and visceral fat are associated with low-grade inflammation (9–11). Because atherogenesis involves low-grade inflammation, it is feasible that similar processes also affect vascular wall stiffening. Indeed, peripheral pulse pressure, a surrogate measure of large artery stiffness, was linked to C-reactive protein (CRP) and IL-6 levels in healthy, middle-aged men (12, 13). However, peripheral pulse pressure does not provide reliable information concerning local conditions throughout the arterial tree, which are known to be differentially affected by aging, metabolic abnormalities, and vasoactive drugs (1, 5, 14).

Several cell types, including activated macrophages, lymphocytes, and smooth muscle cells, as well as adipose cells produce proinflammatory cytokines (11, 15). Thus, the increased levels of circulating inflammatory markers, including IL-6 and TNFα, as found in obese, insulin-resistant, and type 2 diabetic individuals, may reflect inflammation of the arterial wall (11, 15), but may also be derived from adipose tissue, in particular visceral fat (16). Several studies described an association between visceral adiposity and arterial stiffness (8, 17), but the mechanisms by which visceral fat may enhance arterial stiffening have not been clarified. Because visceral obesity is a hallmark of insulin resistance and type 2 diabetes, we hypothesized that the association between arterial stiffness and visceral fat may be mediated by circulating inflammatory markers in patients with uncomplicated type 2 diabetes.

Subjects and Methods

Subjects

Thirty-two subjects, *i.e.* 16 patients with uncomplicated type 2 diabetes and 16 age-matched healthy controls, were studied. Male and

First Published Online December 21, 2004

Abbreviations: BMI, Body mass index; CRP, C-reactive protein; CVD, cardiovascular disease; DC, distensibility coefficient; ECG, electrocardiogram; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; HOMA, homeostasis model assessment method; IR, insulin resistance index; LDL, low-density lipoprotein.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

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postmenopausal female patients, aged 35–65 yr, were selected from general practices after approval from their physicians. Selection criteria were type 2 diabetes of short duration (<5 yr; diagnosed by World Health Organization criteria) (18); no signs, symptoms, or history of CVD; normal electrocardiogram (ECG); body mass index (BMI) less than 28 kg/m^2; seated office blood pressure below 150/90 mm Hg, good metabolic control [glycated hemoglobin (HbA_{1c}), <7.8%]; no smoking; no use of drugs other than sulfonylureas and/or metformin; no insulin use; and no clinical signs of microvascular complications, including albuminuria, retinopathy, and neuropathy. Healthy controls, who had no history or clinical evidence of diabetes or CVD, were recruited through advertisements in local newspapers. During a screening visit, a medical history, physical examination (including standardized measurements of the vibration sense and Ewing's cardiovascular tests) (19), an ECG, and screening laboratory tests were performed on all participants. Microalbuminuria was determined in a 24-h urine collection [albumin/creatinine ratio, 0.7 ± 0.9 mg/mmol in patients, 0.4 ± 0.3 mg/mmol in controls ($P = 0.292$); normal albumin/creatinine ratio, <2.5 mg/mmol]. In patients only, fundus photography was performed, unless a recent (<6 months) written report from the patient's ophthalmologist was available. The protocol was approved by the local ethics committee, and written informed consent was obtained from all participants. The investigation conforms with the principles outlined in the Declaration of Helsinki. After inclusion, the subjects came to the hospital research unit at 0800 h after an overnight fast. On this occasion, venous blood samples were obtained, and the ultrasound examination was performed. During a separate visit, body fat distribution was measured by magnetic resonance imaging. All measurements were finished within 2 wk from the screening visit.

Measurement of carotid artery distensibility by ultrasound

Arterial stiffness was quantified as distensibility of the right common carotid artery. All measurements were performed by the same investigator, using a B-mode ultrasound imager with a 7.5-MHz linear array transducer (Aloka SD 1400, Aloka, Almere, The Netherlands). Subjects were examined in the supine position, with the head tilted slightly to the contralateral side. A three-lead ECG was attached for R-wave triggering. Subsequently, the distal 10 mm of the right carotid artery were visualized, and when an optimal longitudinal ultrasound image was obtained, it was frozen on the R-wave of the ECG and stored on videotape for off-line analysis. In addition, from the same location, continuous B-mode images were recorded for 30 sec. Blood pressure was measured twice, and the mean was taken as the subject's reading. Off-line analysis was performed using dedicated software. In short, on a personal computer screen, the interfaces of the carotid artery were marked over a length of 10 mm during various phases of the cardiac cycle. Distensibility of the carotid artery was determined from digitized images using diameter change during the cardiac cycle (d), the end-diastolic lumen diameter (D), and pulse pressure (PP = systolic − diastolic blood pressure) and was expressed as the distensibility coefficient (DC = (d/D) / PP 10^{-3}/kPa) (20). Distensibility is the inverse of stiffness; a reduced distensibility reflects an increased stiffness. An intraobserver study on distensibility and end-diastolic lumen diameter measurements in 10 healthy subjects showed coefficients of variation of 6.8% and 2.3%, respectively. To determine the presence of subclinical atherosclerosis, carotid intima media thickness was determined off-line according to a previously described protocol by analysis of the B-mode images by an experienced observer using dedicated software (21).

Body fat distribution

General adiposity was assessed as BMI, and distribution of body fat was measured in duplicate with a flexible plastic tape at the level of the abdomen (midway between the lower rib margin and the iliac crest) and the hip (over the greater trochanters) to calculate the waist to hip ratio. Abdominal fat depots (sc and visceral) were quantified by magnetic resonance imaging using a clinical 1.5-T whole body magnetic resonance scanner (Gyroscan ACS/NT15, Philips, Best, The Netherlands). The method was previously described in detail (22). Briefly, patients were scanned in the supine position, and three transverse images were obtained at the abdominal level: the first at the anatomical marker (lower edge of the umbilicus), and one above and one below this position (slice thickness, 10 mm). The same investigator performed all scans, and one blinded, well-trained observer performed image analysis using a dedicated software program (22). The areas of the abdominal fat depots were calculated by converting the number of pixels to square centimeters and taking the average of three abdominal images.

Analytical methods

Hematological parameters, clinical chemistries, and lipids were determined immediately after blood collection at the hospital's routine laboratory. Additional plasma samples were processed and stored at −80 C until assay (IL-6 and CRP). Plasma concentrations of IL-6 (Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands) were determined by ELISA, as described by the manufacturer. The sensitivity of the assay was 0.2–0.4 ng/liter. CRP levels were measured using an ELISA (CRP EIA HS assay, Kordia, The Netherlands; normal range, 0.2–6.0 mg/liter), which was validated previously (23). Hematological parameters were assessed using the Technicon H-1 system (Bayer Diagnostics GmbH, Munich, Germany). Blood glucose concentrations were determined by the hexokinase method on a BM/Hitachi 747 analyzer (Roche, Mannheim, Germany), HbA_{1c} was determined by HPLC after hemolysis (reference range, 4.3–6.3% in nondiabetic subjects; Bio-Rad Laboratories, Richmond, CA). Serum creatinine, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels were determined using an RA 1000 analyzer (Technicon Instruments Corp., Tarrytown, NY). Low-density lipoprotein (LDL) cholesterol levels were calculated with the Friedewald formula. Insulin (reference value, <20 μU/ml) and C-peptide (reference range, 1.2–2.4 ng/ml) were assayed by RIA (Medgenix, Brussels, Belgium). An insulin resistance index (IR) was calculated according to the homeostasis model assessment method (HOMA-IR) (24). Microalbuminuria was measured using immunonephelometry (Array Protein System, Beckman Coulter, Fullerton, CA).

Statistical analysis

Data are expressed as the mean ± SD when normally distributed; nonnormally distributed data are expressed as the median (95% confidence interval and interquartile range). Nonnormally distributed data were log-transformed, and unpaired *t* tests (or, when appropriate, nonparametric tests) were used for comparisons. The Bonferroni method was used to correct for multiple comparisons. Correlations were calculated using Pearson's correlation coefficients. To detect determinants of carotid artery distensibility, univariate and multivariate linear regression analyses were performed. $P < 0.05$ was considered significant. Statistical testing was performed with SPSS version 10.1 (SPSS, Inc., Chicago, IL).

Results

Subject characteristics

Table 1 lists the characteristics of patients and controls. Six of 16 diabetic patients were treated by diet only; the remaining 10 were taking oral blood glucose-lowering drugs, *i.e.* sulfonylurea agents and/or metformin. Patients and controls differed with respect to BMI, HbA_{1c}, fasting plasma glucose and insulin levels, HDL cholesterol, and HOMA-IR; otherwise, they had similar age, blood pressure, heart rate, and plasma concentrations of total cholesterol, LDL cholesterol, and triglycerides. Median self-reported estimated time spent performing moderate exercise (walking, biking, or swimming) was 60 min (range, 30–180 min)/wk, and this was comparable in patients and controls (data not shown).

Carotid artery distensibility, body fat distribution, and markers of inflammation

Mean carotid artery DC was not different in patients and controls (12.1 ± 6.6 *vs.* 14.6 ± 7.7 kPA^{-1}; $P = 0.325$). However,

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TABLE 1. Clinical characteristics of type 2 diabetic and control subjects

Parameter	Patients (n = 16)	Controls (n = 16)
Gender (M/F)	12/4	12/4
Age (yr)	54 ± 8	54 ± 7
Duration of disease (months)	16 (14–36)	NA
BMI (kg/m^2)	25.9 ± 1.6[a]	24.4 ± 1.4
Systolic blood pressure (mm Hg)	135 ± 8	137 ± 11
Diastolic blood pressure (mm Hg)	83 ± 5	83 ± 6
Pulse pressure (mm Hg)	52 ± 11	53 ± 10
Heart rate (beats/min)	69 ± 10	66 ± 11
HbA_{1c} (%)	5.8 ± 1.2[b]	4.9 ± 0.6
Fasting plasma glucose (mg/dl)	140.5 ± 34.2[b]	95.5 ± 9.0
Fasting plasma insulin (mU/liter)	17.8 ± 10.4[b]	9.0 ± 3.4
C-peptide (ng/ml)	3.3 ± 1.2	2.7 ± 2.4
Total cholesterol (mg/dl)	200.8 ± 30.9	208.5 ± 38.6
HDL cholesterol (mg/dl)	42.5 ± 15.4[b]	57.9 ± 11.6
Triglycerides (mg/dl)	141.7 ± 70.9	124.0 ± 88.6
LDL cholesterol (mg/dl)	146.7 ± 30.9	142.9 ± 34.7
HOMA-IR	3.9 (3.7–8.3)[b]	2.1 (1.7–2.6)

Values are the mean ± SD or median (95% confidence interval). NA, Not applicable. Conversion factors for Systeme International units are: total cholesterol, LDL cholesterol, and HDL cholesterol, × 0.0259 (mmol/liter); triglycerides, × 0.01129 (mmol/liter); glucose, × 0.005551 (mmol/liter); insulin, × 7.175 (pmol/liter); C-peptide, × 0.331 (nmol/liter).

[a] $P < 0.02$.

[b] $P < 0.01$.

in diabetic males, carotid DC tended to be lower than in control males (10.9 ± 7.4 *vs.* 16.0 ± 7.4 kPA^{-1}; $P = 0.08$). Mean distension of the carotid artery during the cardiac cycle, d (257.7 ± 141.9 *vs.* 327.5 ± 174.6 μm; $P = 0.222$), as well as the end-diastolic diameter of the carotid artery, D (6401 ± 712 *vs.* 6399 ± 747 μm; $P = 0.996$), were similar in patients and controls. Mean carotid intima media thickness was 0.6993 ± 0.077 mm in the diabetics and 0.6939 ± 0.124 mm in the control subjects ($P = 0.883$). Although mean waist to hip ratios were comparable in patients and controls, diabetic subjects had larger mean visceral fat areas than controls (Table 2; $P < 0.01$), and this difference remained significant after correction for BMI ($P < 0.05$). Mean abdominal sc fat areas were similar in patients and controls (Table 2). Leukocyte counts were significantly higher in patients ($P < 0.05$), whereas the increases in plasma IL-6 and CRP concentrations were not significant (Table 2).

TABLE 2. Body fat distribution, vascular function, and circulating markers of inflammation in patients and controls

Parameter	Patients (n = 16)	Controls (n = 16)
Body fat distribution		
Waist to hip ratio	0.97 ± 0.07	0.97 ± 0.04
Waist circumference (cm)	95.7 ± 5.9	94.1 ± 6.3
Visceral fat area (cm^2)	228 ± 62[a]	173 ± 38
Visceral fat area/height ratio	1.27 ± 0.33[a]	1.00 ± 0.19
Abdominal sc fat area (cm^2)	221 ± 54	194 ± 49
Abdominal sc fat area/height ratio	1.24 ± 0.34	1.11 ± 0.30
Visceral fat/abdominal sc fat ratio	1.10 ± 0.40	0.95 ± 0.30
Markers of inflammation		
Leukocytes (×10^9/liter)	5.9 ± 1.2[b]	4.9 ± 1.0
CRP (mg/liter)	0.9 (0.5–4.8)	0.7 (0.5–1.8)
IL-6 (ng/liter)	1.1 (1.0–3.2)	0.8 (0.7–1.4)

Data are the mean ± SD or median (95% confidence interval).

[a] $P < 0.01$.

[b] $P < 0.05$.

Figure 1 shows scatter plots of the relationship between carotid artery DC and visceral fat area (Fig. 1A), and between DC and concentrations of IL-6 (Fig. 1B) and CRP (Fig. 1C), respectively, in patients and controls. Table 3 lists the correlations between carotid DC in all subjects together as well as in patients and controls separately. When the total study population was analyzed, carotid DC was inversely associated with age ($P = 0.011$), fasting plasma glucose ($P = 0.041$), and visceral fat area ($P = 0.026$); however, the strongest associations were found between DC and CRP ($P < 0.001$) and IL-6 ($P < 0.001$). The associations between carotid DC and leukocytes ($r = -0.287$) and HOMA-IR ($r = -0.263$) were not significant (not shown in Table 3).

In patients, carotid DC was negatively associated with visceral fat area ($P = 0.005$) and plasma CRP levels ($P = 0.002$), but most strongly with plasma IL-6 levels ($P < 0.001$). In addition, significant negative correlations were observed between carotid DC and fasting plasma glucose ($r = -0.560$; $P = 0.019$) and plasma triglyceride levels ($r = -0.606$; $P = 0.01$; data not shown). In controls, carotid DC was inversely correlated with circulating CRP and IL-6 ($P = 0.025$ and $P = 0.020$, respectively), but not with visceral fat area or blood glucose and triglyceride levels (data not shown).

Multivariate models of carotid artery distensibility

Multivariate analysis was performed in the pooled groups to study the association between carotid DC and visceral fat. To this purpose, carotid DC was entered as a dependent variable, and subsequently, visceral fat area, diabetic state, and IL-6 and CRP were entered as independent variables into the model (Table 4). The diabetic state had no effect on the association between DC and visceral fat.

To investigate the possible interaction between visceral fat area or BMI and the diabetic state, interaction terms of these two variables (visceral fat area × diabetic state and BMI × diabetic state) were subsequently introduced into model 2 as an independent variable, but this had no effect on the interaction (all $P > 0.05$; data not shown). When IL-6 was entered into the model, the association between visceral fat and DC disappeared, and a strong inverse relation was observed between carotid DC and IL-6; for every 10% increase in plasma IL-6 concentration, carotid DC decreased by 0.62 kPA^{-1} (model 3). Finally, after adding CRP, the model explained 53.6% of the variance in carotid DC ($P < 0.001$); however, only circulating levels of IL-6 remained significantly related to carotid DC (model 4). Correction for age, mean arterial pressure, HbA_{1c}, fasting plasma glucose, plasma triglycerides, and height did not affect the model, and IL-6 remained significantly associated with carotid DC (data not shown).

Discussion

In the present study, we found an association between carotid artery stiffness, measured as distensibility, and visceral fat area in asymptomatic subjects with uncomplicated, well-controlled type 2 diabetes. However, this association disappeared after correction for plasma IL-6 and CRP, indi-

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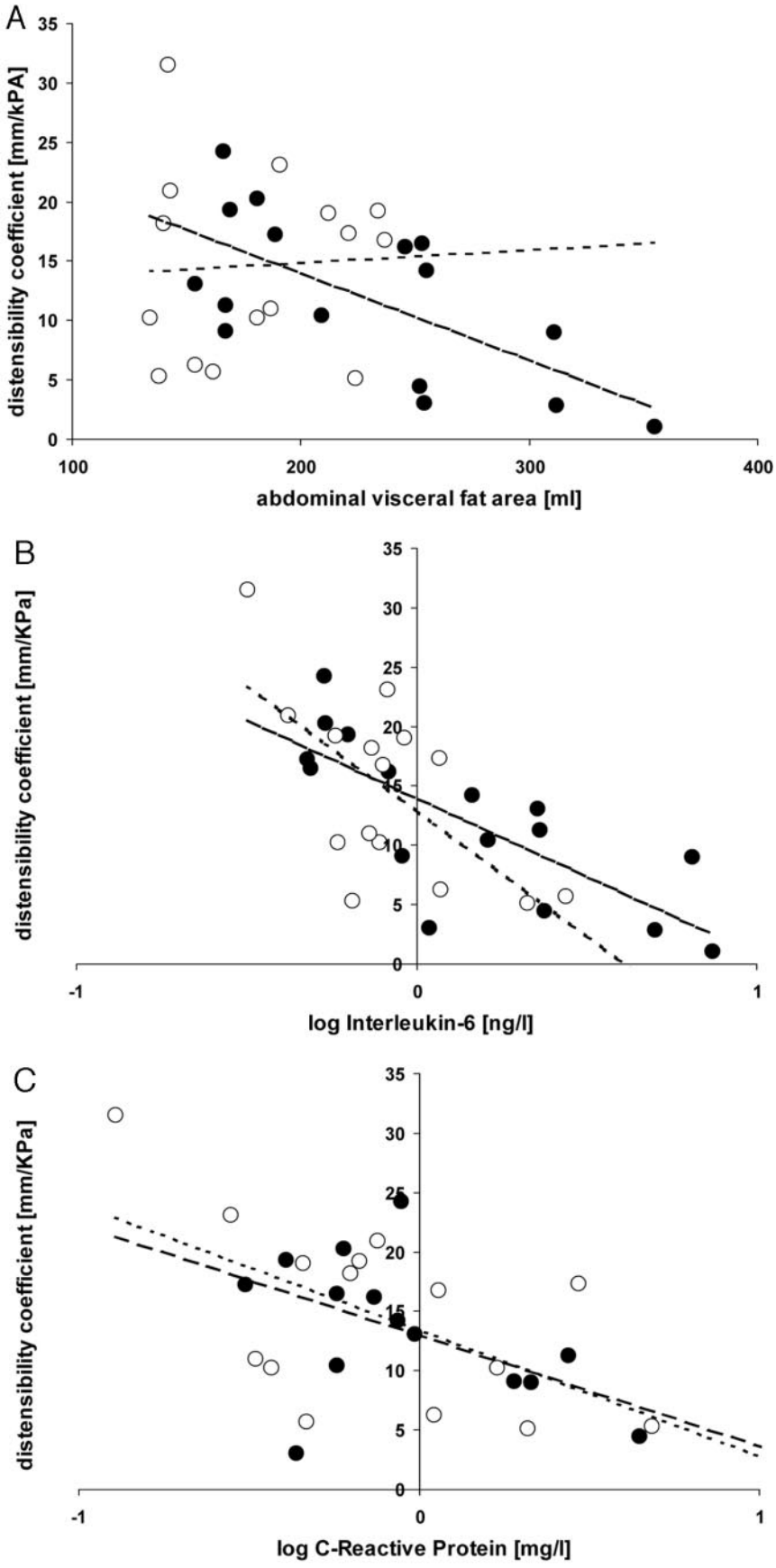

FIG. 1. Scatter plots showing the relationship between common carotid artery DC and visceral fat area (A) and logarithm of IL-6 (B) and of CRP (C) in type 2 diabetic patients (●) and healthy controls (○). —, Regression line in patients; - - -, regression line in controls.

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TABLE 3. Univariate correlations between carotid DC, and age and anthropometric and inflammatory variables

	DC (kPA^{-1})		
	All (r)	Patients (r)	Controls (r)
Age (yr)	−0.445[a]	−0.471	−0.456
BMI (kg/m^2)	−0.329	−0.298	−0.278
Waist circumference (cm)	−0.316	−0.460	−0.213
Systolic blood pressure (mm Hg)	−0.329	−0.373	−0.270
Log CRP (mg/liter)	−0.642[b]	−0.687[b]	−0.576[a]
Log IL-6 (ng/liter)	−0.679[b]	−0.766[b]	−0.591[a]
Visceral fat area (cm^2)	−0.400[a]	−0.660[b]	+0.054

r, Pearson's correlation coefficient.
[a] $P < 0.05$.
[b] $P < 0.01$.

cating that circulating inflammatory markers, which are partially derived from adipose tissue, mediate this relationship.

The association between obesity and arterial stiffness was described previously in nondiabetic populations (25, 26). Subsequently, it was demonstrated that in nondiabetic and diabetic subjects, this relationship was largely driven by visceral fat, which is regarded as a key feature of the metabolic syndrome (8, 17, 27, 28). These studies did not address the mechanisms underlying the associations, but several possible explanations were suggested. Firstly, obesity-associated insulin resistance and the ensuing hyperinsulinemia may promote sodium retention, sympathetic nervous system activation, and induction of vascular smooth muscle cell growth. These mechanisms increase blood pressure and contribute to arterial stiffening. Impaired vascular insulin signaling may reduce nitric oxide bioavailability, resulting in reduction of endothelium-dependent vasodilatation and ultimately in decreased arterial distensibility (5, 29). Blood glucose elevations may also contribute to arterial stiffening by inducing oxidative stress, thus further reducing nitric oxide availability, and by causing glycation of the proteins in the arterial wall (30). Another consequence of insulin resistance that may promote arterial stiffness is the rise in plasma nonesterified fatty acids and the subsequent increase in α-adrenergic reactivity, vascular tone, and blood pressure (29). Secondly, the fat tissue-derived hormone leptin promotes smooth muscle cell proliferation and angiogenesis, and high circulating levels of leptin have been associated with reduced arterial distensibility in healthy adults (31). Finally, adipocytokines may further amplify the inflammatory cascade by stimulating hepatic CRP synthesis and altering endothelial functional properties, resulting in vascular wall inflammation and elastin degradation (11, 15, 32). The exact role of the adipose tissue as mediator of systemic and vessel wall inflammation, however, remains unclear, because multiple cell types produce many inflammatory substances (15). Moreover, recent evidence indicates that macrophage infiltration of adipose tissue increases in obesity and that these macrophages are an important source of inflammatory cytokines previously believed to be secreted by adipocytes from biopsies that invariably contained contaminations of multiple cell types (33).

In patients with active systemic inflammatory disease, including systemic vasculitis, rheumatoid arthritis, and systemic lupus erythematosus, increased arterial stiffness was observed (34–38), which some researchers found associated

TABLE 4. Multivariate associations between carotid artery DC and visceral fat area

	Δ DC (kPA^{-1}) (95% CI)	P^a	r	r^2	P^b
Model 1			0.400	0.160	0.026
Visceral fat area (/10 cm^2)	−0.52 (−0.97 to −0.07)	0.026			
Model 2 (model 1 + diabetic state)			0.400	0.160	0.087
Visceral fat area (/10 cm^2)	−0.51 (−0.10 to 0.00)	0.049			
Diabetic state	−0.21 (−5.86 to 5.43)	0.939			
Model 3 (model 2 + IL-6)			0.683	0.466	0.001
Visceral fat area (/10 cm^2)	0.05 (−0.36 to 0.62)	0.830			
Diabetic state	0.42 (−4.02 to 4.74)	0.854			
10% increase in IL-6 (ng/liter)	−0.62 (−0.95 to −0.30)	0.001			
Model 4 (model 3 + CRP)			0.732	0.536	<0.001
Visceral fat area (/10 cm^2)	0.13 (−0.36 to 0.62)	0.596			
Diabetic state	0.36 (−4.02 to 4.74)	0.868			
10% increase in IL-6 (ng/liter)	−0.45 (−0.81 to −0.09)	0.017			
10% increase in CRP (mg/liter)	−0.23 (−0.47 to 8.49)	0.058			
Model 5 (model 3): association between DC and IL-6 after correction for each of following variables					
a) Age	−0.43 (−0.80 to −0.06)	0.025	0.736	0.542	0.001
b) Gender	−0.44 (−0.81 to −0.07)	0.022	0.734	0.539	0.001
c) Height	−0.42 (−0.81 to −0.03)	0.035	0.736	0.540	0.001
d) BMI	−0.44 (−0.82 to −0.07)	0.022	0.733	0.537	0.001
e) Mean arterial pressure	−0.44 (−0.80 to −0.07)	0.021	0.740	0.548	0.001
f) HbA_{1c}	−0.44 (−0.78 to −0.11)	0.012	0.784	0.614	0.001
g) Fasting plasma glucose	−0.43 (−0.80 to −0.07)	0.020	0.745	0.554	0.001
h) Plasma triglycerides	−0.45 (−0.82 to −0.08)	0.019	0.732	0.536	0.001

CI, Confidence interval; r and r^2, for the respective models, *i.e.* in model 1, with DC as dependent and visceral fat area as independent variable; in model 2, visceral fat area and diabetic state are independent variables; in model 3, visceral fat area, diabetic state, and log [IL-6] are independent variables; in model 4, visceral fat area, diabetic state, log [IL-6] and log [CRP] are independent variables. Models 1–4 are uncorrected for other cardiovascular risk factors or components of the metabolic syndrome. In models 5 (a–h), the possible confounders are separately entered for correction.
[a] Level of significance for the association between DC and the separate components of the model.
[b] Level of significance of the model.

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with circulating inflammatory markers (37), whereas others did not (35, 38). This association was observed in patients with active disease, who had significantly elevated circulating levels of inflammatory markers, but in subjects with disease in remission, whose CRP and IL-6 levels had dropped to control levels, the association disappeared (34). We found that the low-grade inflammatory status in our patients, whose plasma IL-6 and CRP levels were within the normal range, may also play a role in arterial stiffening. These findings were confirmed in recent studies showing an association between normal CRP plasma levels and arterial stiffness in healthy subjects and patients with hypercholesterolemia (39, 40).

How can the observed interrelation among visceral fat, plasma IL-6 and CRP, and arterial stiffness in our patients be reconciled? As pointed out, the exact source of plasma IL-6 in patients remains unknown, but adipose tissue may be responsible for about 30% of the circulating IL-6 concentrations under basal conditions (11), with visceral fat being more active than sc fat with regard to cytokine synthesis (16). Circulating CRP is mainly derived from the liver, and IL-6 is regarded as a major stimulus of hepatic CRP production (12, 41). Conversely, CRP may stimulate IL-6 production from endothelial cells, thus promoting the inflammatory cascade (15). Although this study did not explore the mechanisms by which inflammation affects arterial stiffening, we postulate that IL-6, regardless of its origin, stimulates hepatic CRP production and that both inflammatory mediators contribute to vascular wall inflammation, endothelial dysfunction, and structural changes leading to vascular stiffening.

Our study has several limitations. Firstly, the number of patients is limited; however, they constitute a relatively homogenous group due to the strict inclusion criteria. Although strict selection limits generalization of the data, the demonstration of significant associations in this small population supports the relevance of the findings. Secondly, the associations found do not reflect causal relationships. Finally, the observed interrelations among visceral fat, inflammatory markers, and arterial stiffness only concern the elastic common carotid artery and may not be extrapolated to other places of the vascular tree, in particular muscular arteries (1, 42).

In conclusion, we found an association between carotid artery stiffness and visceral fat area in patients with well-controlled, uncomplicated, type 2 diabetes. However, this relationship was strongly attenuated and disappeared when adjustments were made for circulating levels of IL-6 and CRP, both of which were within the normal range. Future studies should explore the origin of the circulating inflammatory markers and the mechanisms by which they, even at low plasma concentrations, promote arterial stiffening in type 2 diabetes.

Acknowledgments

Received August 9, 2004. Accepted December 13, 2004.

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