

# Arterial Stiffness Is Increased in Patients With Type 1 Diabetes Without Cardiovascular Disease

A potential role of low-grade inflammation

GEMMA LLURADÓ, MD<sup>1</sup>

VICTÒRIA CEPERUELO-MALLAFRÉ, MD, PHD<sup>2,3</sup>

CARME VILARDELL, MD, PHD<sup>4</sup>

RAFAEL SIMÓ, MD, PHD<sup>5</sup>

NÚRIA FREIXENET, MD<sup>1</sup>

JOAN VENDRELL, MD, PHD<sup>3</sup>

JOSÉ MIGUEL GONZÁLEZ-CLEMENTE, MD, PHD<sup>1</sup>

**OBJECTIVE**—To investigate the relationship between arterial stiffness and low-grade inflammation in subjects with type 1 diabetes without clinical cardiovascular disease.

**RESEARCH DESIGN AND METHODS**—Sixty-eight patients with type 1 diabetes and 68 age- and sex-matched healthy subjects were evaluated. Arterial stiffness was assessed by aortic pulse wave velocity (aPWV). Serum concentrations of high-sensitivity C-reactive protein (hsCRP), interleukin (IL)-6, and soluble fractions of tumor necrosis factor- $\alpha$  receptors 1 and 2 (sTNF $\alpha$ R1 and sTNF $\alpha$ R2, respectively) were measured. All statistical analyses were stratified by sex.

**RESULTS**—Subjects with diabetes had a higher aPWV compared with healthy control subjects (men: 6.9 vs. 6.3 m/s,  $P < 0.001$ ; women: 6.4 vs. 6.0 m/s,  $P = 0.023$ ). These differences remained significant after adjusting for cardiovascular risk factors. Men with diabetes had higher concentrations of hsCRP (1.2 vs. 0.6 mg/L;  $P = 0.036$ ), IL-6 (0.6 vs. 0.3 pg/mL;  $P = 0.002$ ), sTNF $\alpha$ R1 (2,739 vs. 1,410 pg/mL;  $P < 0.001$ ), and sTNF $\alpha$ R2 (2,774 vs. 2,060 pg/mL;  $P < 0.001$ ). Women with diabetes only had higher concentrations of IL-6 (0.6 vs. 0.4 pg/mL;  $P = 0.039$ ). In men with diabetes, aPWV correlated positively with hsCRP ( $r = 0.389$ ;  $P = 0.031$ ) and IL-6 ( $r = 0.447$ ;  $P = 0.008$ ), whereas in women with diabetes no significant correlation was found. In men, multiple linear regression analysis showed that the following variables were associated independently with aPWV: age, BMI, type 1 diabetes, and low-grade inflammation ( $R^2 = 0.543$ ). In women, these variables were age, BMI, mean arterial pressure, and type 1 diabetes ( $R^2 = 0.550$ ).

**CONCLUSIONS**—Arterial stiffness assessed as aPWV is increased in patients with type 1 diabetes without clinical cardiovascular disease, independently of classical cardiovascular risk factors. In men with type 1 diabetes, low-grade inflammation is independently associated with arterial stiffness.

*Diabetes Care* 35:1083–1089, 2012

It is well established that type 2 diabetes is a risk factor for cardiovascular disease. However, it is less well known that the relative risk of cardiovascular

disease in type 1 diabetes can be as much as 10-fold greater than in the healthy population, especially in women (1), being even greater than in type 2 diabetes (2).

From the <sup>1</sup>Department of Diabetes, Endocrinology, and Nutrition, Hospital of Sabadell, Corporació Sanitària i Universitària Parc Taulí (Universitat Autònoma de Barcelona), Sabadell, Spain; the <sup>2</sup>Centro de Investigación Biomédica en Red-Fisiopatología de la Obesidad y la Nutrición (CIBERObn), Hospital Clínico Virgen de la Victoria, Málaga, Spain; the <sup>3</sup>Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Hospital Universitari Joan XXIII de Tarragona, Institut Pere Virgili, Universitat Rovira i Virgili, Tarragona, Spain; the <sup>4</sup>Diabetes, Endocrinology, and Nutrition Unit, Hospital Sant Joan de Déu de Manresa, Xarxa Assistencial Althaia, Manresa, Spain; and the <sup>5</sup>Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM) and Diabetes and Metabolism Research Unit, Institut de Recerca Hospital Universitari Vall d'Hebrón (VHIR), Universitat Autònoma de Barcelona, Barcelona, Spain.

Corresponding author: José Miguel González-Clemente, josmi.gonza@gmail.com.

Received 22 August 2011 and accepted 6 January 2012.

DOI: 10.2337/dc11-1475

© 2012 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See <http://creativecommons.org/licenses/by-nc-nd/3.0/> for details.

Consequently, cardiovascular disease is the major cause of mortality in type 1 diabetes (2). Diabetes results in an accelerated arteriosclerotic process, which is not fully explained by classical cardiovascular risk factors. As a result, the pathophysiological mechanisms underlying cardiovascular events in type 1 diabetes are not completely understood.

Arterial stiffness is an early sign of arteriosclerosis (3), and its study would be appropriate for investigating the arteriosclerotic mechanisms long before any cardiovascular event occurs. Arterial stiffness predicts cardiovascular events independently of classical cardiovascular risk factors in several populations (see below). Therefore, it can be assumed that it reflects the deleterious effect of all cardiovascular risk factors (known and unknown) on the arterial wall. The gold standard for measuring central arterial stiffness is aortic pulse wave velocity (aPWV), according to a recent consensus (4). aPWV independently predicts cardiovascular events and mortality in the general population, in the elderly, in hypertensive individuals, in subjects with end-stage renal failure, and in subjects with type 2 diabetes (5).

Finally, little is known regarding factors involved in the pathophysiology of arterial stiffness in type 1 diabetes. One of these factors could be low-grade inflammation. High-sensitivity C-reactive protein (hsCRP) is the most established downstream marker of low-grade inflammation and has been reported to be a strong predictor of cardiovascular outcomes (6). The primary proinflammatory cytokines interleukin (IL)-6 and tumor necrosis factor (TNF)- $\alpha$  are the main inducers for the hepatic synthesis of hsCRP (7). Although there is less evidence in comparison with hsCRP, both of them also have been associated with the prediction of cardiovascular outcomes (8–10). Low-grade inflammation also has been associated with the development of both micro- and macrovascular complications in type 1 diabetes (11). Indeed, low-grade inflammation impairs endothelial function and has been associated

with an increase in aPWV in healthy subjects (12), in hypertensive individuals (13), and in subjects with chronic kidney disease (14) or with metabolic syndrome (15). No such evidence exists in type 1 diabetes; however, recently an activation of the TNF $\alpha$  system has been reported in association with an increase in brachial PP, a surrogate marker of arterial stiffness, in normotensive subjects with type 1 diabetes (16).

Our main objective was to evaluate aPWV as a measure of arterial stiffness in a group of subjects with type 1 diabetes without clinical cardiovascular disease and to explore its relationship with biomarkers of low-grade inflammation. Because the role of low-grade inflammation in the atherosclerotic process seems to be different in men and women (17), our study was stratified by sex, and the sample size was calculated taking this stratification into account.

## RESEARCH DESIGN AND METHODS

—Sixty-eight patients with type 1 diabetes (34 men and 34 women), aged 18–65 years, and 68 age- and sex-matched healthy subjects were included in our study. None of them had any condition associated with an inflammatory response (e.g., acute or chronic inflammatory infectious diseases) or had received anti-inflammatory treatment in the previous 6 months. None of them had any clinical cardiovascular disease. Subjects with type 1 diabetes were consecutively recruited from our outpatient clinic, and all had at least 1 year of duration/evolution of diabetes. The control group was recruited from hospital staff members and their relatives and friends.

After an overnight fast, venous blood samples were taken, and aliquots of plasma and serum were stored at  $-80^{\circ}\text{C}$  until processing. In women, all measurements were conducted during the follicular phase of the menstrual cycle. The following information was recorded using a predefined standardized form: sex, age, diabetes duration, BMI, waist-to-hip ratio, systolic and diastolic blood pressure (SBP and DBP, respectively), and mean arterial pressure (MAP; defined as  $1/3\text{ SBP} + 2/3\text{ DBP}$  — physical activity [International Physical Activity Questionnaire], cigarette smoking, alcohol intake, insulin dose or the use of any other medical treatment, HbA<sub>1c</sub>, lipid profile, serum concentrations of hsCRP, IL-6, soluble fractions of the TNF $\alpha$  receptors 1 and 2 [sTNF $\alpha$ R1 and sTNF $\alpha$ R2, respectively],

and microvascular complications [only in the cases]). Hypertension was defined as having blood pressure  $>140/90$  mmHg (18) and/or being under antihypertensive treatment. Dyslipidemia was defined as having concentrations of total cholesterol  $>5.2$  mmol/L, triglycerides  $>1.7$  mmol/L, HDL cholesterol  $<1.03$  mmol/L, LDL cholesterol  $>3.4$  mmol/L (19), and/or receiving drug treatment for dyslipidemia.

The study protocol was approved by our hospital's ethics committee and was conducted according to the principles of the Declaration of Helsinki. All subjects gave their informed consent before participating in the study.

## Assessment of microvascular complications

Peripheral polyneuropathy was assessed through a previously described two-step protocol combining the 15-item Michigan Neuropathy Screening Instrument and a physical examination evaluation (16). Retinopathy was classified according to the data from our department database. Subjects were classified into the following three groups according to the degree of retinopathy: no retinopathy, nonproliferative retinopathy, or proliferative retinopathy. Nephropathy was evaluated by the measurement of urinary albumin excretion. Subjects with a urinary albumin-to-creatinine ratio  $>3.4$  mg/mmol (20), or those who previously were treated with converting enzyme inhibitors or angiotensin receptor blockers (for microalbuminuria or macroalbuminuria), were considered as having diabetic nephropathy.

## Assessment of arterial stiffness

**Measurement of aPWV.** We measured brachial blood pressure three times with the subjects in a sitting position; the mean of the last two measurements was used in all calculations. Subjects rested in the supine position, and measurements were taken immediately after the determination of blood pressure in accordance with the recommendations of the recent consensus on arterial stiffness (4). Subjects were asked to refrain from smoking and from eating or taking caffeine beverages at least 3 h before measurements. aPWV was determined by sequential applanation tonometry (Millar tonometer, SPC-301; Millar Instruments, Houston, TX) at the carotid and femoral arteries gated to a three-lead electrocardiogram using the SphygmoCor device (SphygmoCor; AtCor, Sydney, Australia). Time delay was calculated

using a foot-of-the-wave method. The surface distance from the suprasternal notch to each recording site was measured. The total transit distance was calculated by subtracting the sternal notch to carotid distance from the sternal notch to femoral distance. aPWV was calculated using the total transit distance divided by the time delay. aPWVs not achieving the automatic quality controls specified by the SphygmoCor software were rejected. The mean of two aPWV measurements was taken for each subject for all calculations. Data were available for all the participants included in the study.

## Laboratory analyses

HbA<sub>1c</sub> was determined by high-performance liquid chromatography (Menarini Diagnostics, Firenze, Italy). Total serum cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol were measured using standard enzymatic methods. hsCRP was determined by immunonephelometry (Siemens, Munich, Germany). IL-6 was determined by enzyme-linked immunosorbent assay (R&D Systems, Oxon, U.K.) as were sTNF $\alpha$ R1 (Hycultbiotech, Uden, The Netherlands) and sTNF $\alpha$ R2 (R&D Systems).

## Statistical analyses

We calculated that the number of subjects needed to find a difference of 0.5 m/s in aPWV between men and women with type 1 diabetes and their respective control subjects would be 34 in each of the four groups ( $\alpha = 0.05$  and  $\beta = 20\%$ ). Data are presented as percentages, means (SD) for variables normally distributed, or medians (interquartile range) for variables not normally distributed. All data were tested for normality using the Kolmogorov-Smirnov test. To improve skewedness and kurtosis, variables not normally distributed were log transformed. The analyses were performed stratified by sex. Differences between patients with type 1 diabetes and control subjects were analyzed using the  $\chi^2$  test for comparisons of proportions and unpaired *t* tests or Mann-Whitney *U* tests for comparisons of quantitative variables, as needed. In both men and women, we assessed the potential relationships between arterial stiffness and all inflammatory-related serum proteins evaluated through univariate, nonparametric correlations and linear regression models to adjust for potential confounders. Variables for linear regression analyses were selected based on univariate correlation analyses

and those variables known or likely to be associated with arterial stiffness. In the final model, the variables included for both sexes were age, smoking status, physical activity, hypertension (no/yes), dyslipidemia (no/yes), BMI, MAP, total cholesterol, log triglycerides, logHDL cholesterol, type 1 diabetes, and low-grade inflammation. Because inflammatory-related serum proteins only were measured once, the association (if any) of low-grade inflammation with arterial stiffness would tend to be underestimated. To address this issue, a *z* score was calculated for each inflammatory-related serum protein evaluated as the following: (value in the individual – mean value in the study population)/SD. Subsequently, the low-grade inflammation general score was calculated as the following: (*z* score of hsCRP + *z* score of IL-6 + *z* score of sTNF $\alpha$ R1 + *z* score of sTNF $\alpha$ R2)/4. In addition, it is reasonable to consider that the integrated information obtained using these four selected proinflammatory markers is better than if we had used each parameter separately. The IBM SPSS Statistics (version 19 for Macintosh; IBM, Armonk, NY) was used for all calculations. All *P* values were two sided, and a *P* value <0.05 was considered statistically significant.

**RESULTS**—We evaluated 68 patients with type 1 diabetes and 68 age- and sex-matched healthy subjects (*n* = 136). Their clinical and analytical characteristics are shown in Table 1 for men and Table 2 for women. Of 136 subjects, 8 were on antihypertensive drugs (7 patients and 1 control subject), 15 were on statins (14 patients), and 6 were on antiplatelet drugs (all with diabetes). A total of 13 patients were on levothyroxine treatment (8 with diabetes; range dose 25–150  $\mu$ g/day), but all had normal serum concentrations of thyroxine and thyrotropin.

Subjects with type 1 diabetes, compared with healthy control subjects, showed higher values of fasting plasma glucose and HbA<sub>1c</sub>. Men with type 1 diabetes were more hypertensive than control subjects and had higher values of SBP. Women with type 1 diabetes presented higher BMI values. Subjects with type 1 diabetes presented a better nonsignificant lipid profile than control subjects, probably as a result of the significant number of patients treated with statins.

Subjects with type 1 diabetes (men and women) had a higher aPWV compared with their respective control subjects (men: 6.9 m/s [6.5–7.9] vs. 6.3 m/s

**Table 1—Clinical characteristics of study population (men)**

Men	Healthy control subjects	Type 1 diabetic subjects	<i>P</i>
<i>n</i>	34	34	
Age (years)	35.6 (9.0)	36.5 (8.9)	0.963
Alcohol intake (g/day)	2.9 (0–7.1)	5.7 (2.9–15.0)	0.008
Current smokers [n (%)]	7 (20.6)	12 (35.3)	0.089
Physical activity (METS min/week)	1,395.0 (779.6–2,265.8)	1,903.0 (910.5–2,776.5)	0.270
Family history of coronary heart disease [n (%)]	2 (5.9)	2 (5.9)	1.000
Family history of type 2 diabetes [n (%)]	5 (14.7)	7 (20.6)	0.525
Family history of type 1 diabetes [n (%)]	0 (0)	2 (5.9)	0.493
Hypertension [n (%)]	3 (8.8)	13 (38.2)	0.004
Dyslipidemia [n (%)]	17 (50.0)	18 (52.9)	0.808
Diabetes duration (years)	—	14.00 (8.50–20.50)	—
Microvascular complications [n (%)]	—	9 (26.5)	—
Retinopathy [n (%)]	—	5 (14.7)	—
None [n (%)]	—	29 (85.3)	—
Nonproliferative [n (%)]	—	4 (11.8)	—
Proliferative [n (%)]	—	1 (2.9)	—
Nephropathy [n (%)]	—	6 (17.6)	—
Peripheral polyneuropathy [n (%)]	—	0 (0)	—
BMI (kg/m <sup>2</sup> )	25.0 (2.8)	26.1 (3.3)	0.138
Waist (cm)	91.0 (10.1)	90.6 (11.3)	0.862
Waist-to-hip ratio	0.90 (0.10)	0.91 (0.07)	0.532
SBP (mmHg)	124.9 (9.5)	131.7 (10.8)	0.008
DBP (mmHg)	73.8 (8.0)	76.7 (7.0)	0.124
MAP (mmHg)	90.8 (8.1)	95.0 (7.3)	0.029
Fasting plasma glucose (mmol/L)	4.76 (0.57)	8.71 (3.76)	<0.001
Total cholesterol (mmol/L)	5.08 (1.54)	4.74 (0.82)	0.260
Triglycerides (mmol/L)	0.88 (0.69–1.29)	0.83 (0.69–1.14)	0.585
HDL cholesterol (mmol/L)	1.31 (1.13–1.49)	1.31 (1.11–1.76)	0.560
LDL cholesterol (mmol/L)	2.81 (2.26–3.59)	2.61 (2.23–3.13)	0.213
HbA <sub>1c</sub> (%)	5.4 (5.1–5.5)	7.3 (6.6–7.9)	<0.001
Urinary albumin-to-creatinine ratio (mg/mmol)	0.39 (0.30–0.51)	0.28 (0.20–0.47)	0.125
aPWV (m/s)	6.3 (5.7–6.7)	6.9 (6.5–7.9)	<0.001
hsCRP (mg/L)	0.6 (0.3–1.1)	1.2 (0.5–2.9)	0.036
IL-6 (pg/mL)	0.3 (0.2–0.6)	0.6 (0.3–1.0)	0.002
sTNF $\alpha$ R1 (pg/mL)	1,410 (1,113–2,308)	2,739 (1,748–3,224)	<0.001
sTNF $\alpha$ R2 (pg/mL)	2,060 (1,870–2,365)	2,774 (2,267–3,064)	<0.001

Data are percentages, means (SD), or medians (interquartile range), unless otherwise indicated.

[5.7–6.7], *P* < 0.001; women: 6.4 m/s [5.9–7.5] vs. 6.0 m/s [5.3–6.7], *P* = 0.023). These differences remained significant after adjusting for classical cardiovascular risk factors (age, physical activity, smoking status, hypertension, dyslipidemia, and BMI) in both sexes (men: *P* = 0.001; women: *P* = 0.025). Men with type 1 diabetes showed higher serum concentrations of hsCRP, IL-6, sTNF $\alpha$ R1, and sTNF $\alpha$ R2 (Table 1). Women with type 1 diabetes only had higher concentrations of IL-6 (Table 2).

In type 1 diabetes, univariate correlations showed that aPWV correlated positively with age, BMI, waist, waist-to-hip ratio, SBP, MAP, and total and LDL cholesterol (Table 3). In healthy subjects, aPWV was associated with age, BMI, waist, total and LDL cholesterol, and fasting plasma glucose. In men with type 1 diabetes, aPWV correlated positively with hsCRP (*r* = 0.389; *P* = 0.031) and IL-6 (*r* = 0.447; *P* = 0.008). However, no significant association between aPWV and inflammatory-related serum proteins was

Table 2—Clinical characteristics of study population (women)

Women	Healthy control subjects	Type 1 diabetic subjects	P
n	34	34	
Age (years)	35.3 (11.4)	35.2 (11.2)	0.971
Alcohol intake (g/day)	1.4 (0–2.9)	0.0 (0.0–0.7)	0.001
Current smokers [n (%)]	9 (26.5)	12 (35.3)	0.536
Physical activity (METs min/week)	1,386.0 (770.3–2,079.0)	1,386.0 (672.8–1,686.0)	0.442
Family history of coronary heart disease [n (%)]	4 (11.8)	1 (2.9)	0.356
Family history of type 2 diabetes [n (%)]	7 (20.6)	9 (26.5)	0.567
Family history of type 1 diabetes [n (%)]	1 (2.9)	3 (8.8)	0.614
Hypertension [n (%)]	0 (0)	4 (11.8)	0.114
Dyslipidemia [n (%)]	17 (50)	14 (41.2)	0.465
Diabetes duration (years)	—	12.00 (6.75–18.00)	—
Microvascular complications [n (%)]	—	7 (20.6)	—
Retinopathy [n (%)]	—	5 (14.7)	—
None [n (%)]	—	29 (85.3)	—
Nonproliferative [n (%)]	—	2 (5.9)	—
Proliferative [n (%)]	—	3 (8.8)	—
Nephropathy [n (%)]	—	3 (8.8)	—
Peripheral polyneuropathy [n (%)]	—	0 (0)	—
BMI (kg/m <sup>2</sup> )	23.0 (3.1)	25.3 (3.9)	0.009
Waist (cm)	76.3 (6.9)	80.0 (10.3)	0.092
Waist-to-hip ratio	0.80 (0.06)	0.81 (0.07)	0.529
SBP (mmHg)	116.3 (9.5)	118.3 (9.6)	0.379
DBP (mmHg)	67.9 (7.8)	69.1 (7.9)	0.508
MAP (mmHg)	84.0 (7.9)	85.5 (7.5)	0.417
Fasting plasma glucose (mmol/L)	4.59 (0.48)	9.59 (3.55)	<0.001
Total cholesterol (mmol/L)	5.18 (1.11)	4.82 (0.92)	0.146
Triglycerides (mmol/L)	0.72 (0.56–0.93)	0.70 (0.53–0.84)	0.484
HDL cholesterol (mmol/L)	1.77 (1.46–1.99)	1.80 (1.49–2.20)	0.377
LDL cholesterol (mmol/L)	2.78 (2.18–3.48)	2.47 (1.90–2.97)	0.056
HbA <sub>1c</sub> (%)	5.3 (5.2–5.4)	7.8 (7.1–9.1)	<0.001
Urinary albumin-to-creatinine ratio (mg/mmol)	0.38 (0.27–0.65)	0.47 (0.30–0.91)	0.315
aPWV (m/s)	6.0 (5.3–6.7)	6.4 (5.9–7.5)	0.023
hsCRP (mg/L)	0.9 (0.4–2.8)	1.4 (0.7–2.5)	0.447
IL-6 (pg/mL)	0.4 (0.2–0.6)	0.6 (0.3–1.2)	0.039
sTNF $\alpha$ R1 (pg/mL)	1,917 (1,355–3,295)	2,262 (1,366–2,978)	0.864
sTNF $\alpha$ R2 (pg/mL)	2,215 (1,897–2,700)	2,295 (2,018–3,006)	0.320

Data are percentages, means (SD), or medians (interquartile range), unless otherwise indicated.

found in healthy control subjects (men and women) or in women with type 1 diabetes. In women with type 1 diabetes, aPWV correlated positively with diabetes duration ( $r = 0.538$ ;  $P = 0.001$ ). No association between aPWV and chronic diabetes complications was found either in men or women.

To evaluate the main predictors of aPWV, multiple linear regression analyses were performed. Inflammatory-related serum proteins were tested in all these

models as well as the low-grade inflammation general score previously described. In men, the best multiple linear regression model showed that the independent predictors of aPWV were age, BMI, type 1 diabetes, and the low-grade inflammation general score ( $R^2 = 0.543$ ;  $P < 0.001$ ). In women, age, BMI, MAP, and type 1 diabetes were the independent predictors of aPWV ( $R^2 = 0.550$ ;  $P < 0.001$ ) (Table 4). Even after adjusting for metabolic control (logHbA<sub>1c</sub>) these results did not change.

**CONCLUSIONS**—The main finding of the current study is that arterial stiffness (assessed as aPWV) is increased in subjects with type 1 diabetes compared with age- and sex-matched healthy subjects, even after controlling for classic cardiovascular risk factors. Of note is the fact that our study suggests an association, for the first time, between arterial stiffness and low-grade inflammation in men with type 1 diabetes.

Our results confirm, and reinforce in a larger population, previous studies showing an increase in arterial stiffness assessed as aPWV in adult subjects with type 1 diabetes when compared with healthy subjects (21,22). We also found that patients with type 1 diabetes have higher concentrations of inflammatory-related serum proteins than healthy control subjects, as previously reported in several studies (23,24). Men with type 1 diabetes had higher concentrations of hsCRP, IL-6, sTNF $\alpha$ R1, and sTNF $\alpha$ R2 than their control subjects. However, women only showed differences for IL-6. Despite these discrepancies, we found no differences between sexes within the same group. Previous studies have found higher inflammatory parameters in type 1 diabetic women than in men (24–26). Nevertheless, other authors have failed to report such differences, which agrees with our results (27–29).

Our study shows, for the first time, an association between arterial stiffness and low-grade inflammation in subjects with type 1 diabetes. We have observed that men with type 1 diabetes have higher aPWV and higher concentrations of inflammatory-related serum proteins than control subjects. When we adjusted these data, a general score of low-grade inflammation was an independent predictor of aPWV, taking into account diabetes status. Similar associations between aPWV and inflammatory-related serum proteins have been previously reported in healthy individuals (12), in hypertensive subjects (13), in subjects with chronic kidney disease (14), and in individuals with metabolic syndrome (15) for both sexes. However, we only observed this association in men. Tsioufis et al. (30) reported similar results in hypertensive patients. They found that hsCRP and adiponectin were independent predictors of aPWV only in men. Colhoun et al. (31) reported that hsCRP was independently associated with coronary artery calcification (a validated measure of coronary atherosclerosis) only in men with type 1 diabetes.

**Table 3—Spearman correlation coefficients for the association between aPWV and cardiovascular risk factors and low-grade inflammation (stratified for diabetes status and sex)**

	Men		Women	
	Healthy	Type 1 diabetes	Healthy	Type 1 diabetes
Age (years)	0.598 (<0.001)	0.515 (0.003)	0.577 (0.005)	0.621 (<0.001)
Smoking (no/yes)	0.248 (0.158)	0.113 (0.537)	0.266 (0.128)	−0.152 (0.391)
Physical activity	−0.257 (0.142)	0.077 (0.677)	0.262 (0.354)	−0.004 (0.983)
BMI (kg/m <sup>2</sup> )	0.439 (0.009)	0.515 (0.002)	0.542 (0.001)	0.412 (0.015)
Waist (cm)	0.461 (0.006)	0.567 (<0.001)	0.620 (0.001)	0.541 (0.001)
Waist-to-hip ratio	0.436 (0.010)	0.412 (0.016)	0.213 (0.227)	0.385 (0.024)
SBP (mmHg)	0.249 (0.156)	0.364 (0.037)	0.158 (0.371)	0.528 (0.001)
DBP (mmHg)	0.282 (0.106)	0.279 (0.122)	0.235 (0.182)	0.490 (0.003)
MAP (mmHg)	0.275 (0.115)	0.3470 (0.009)	0.280 (0.109)	0.547 (0.001)
Cholesterol (mmol/L)	0.534 (0.001)	0.498 (0.004)	0.404 (0.018)	0.393 (0.021)
Triglycerides (mmol/L)	0.498 (0.003)	0.017 (0.922)	0.259 (0.139)	−0.113 (0.525)
HDL (mmol/L)	0.019 (0.914)	−0.70 (0.695)	0.154 (0.384)	0.323 (0.062)
LDL (mmol/L)	0.578 (<0.001)	0.460 (0.009)	0.366 (0.033)	0.400 (0.019)
Fasting plasma glucose (mmol/L)	0.465 (0.006)	0.153 (0.389)	0.380 (0.026)	0.137 (0.440)
HbA <sub>1c</sub> (%)	0.218 (0.215)	0.177 (0.316)	0.328 (0.059)	0.048 (0.786)
hsCRP (mg/L)	0.010 (0.958)	0.389 (0.031)	0.203 (0.249)	0.037 (0.836)
IL-6 (pg/mL)	0.125 (0.482)	0.447 (0.008)	0.225 (0.202)	−0.183 (0.316)
sTNF $\alpha$ R1 (pg/mL)	0.264 (0.167)	0.204 (0.298)	0.255 (0.145)	0.077 (0.664)
sTNF $\alpha$ R2 (pg/mL)	0.070 (0.694)	0.163 (0.407)	0.295 (0.107)	0.224 (0.203)
Low-grade inflammation score	0.248 (0.179)	0.369 (0.035)	0.321 (0.083)	0.051 (0.776)

Data are Spearman correlation coefficients (*P* value).

These results indicate that more studies are needed to elucidate the potential gender differences in the pathophysiology of cardiovascular disease in type 1 diabetes.

The exact mechanisms responsible for the increase in arterial stiffness in type 1 diabetes are not fully understood but are likely to reflect a complex interaction

between structural and functional changes in the arterial wall. The structural changes are characterized by an overproduction of abnormal collagen and diminished quantities of normal elastin (32). Our results suggest that low-grade inflammation could play a role in the increase of arterial stiffness in type 1 diabetes. However, other mechanisms, such as the accumulation of advanced glycation end products and endothelial dysfunction, also could be involved (32).

Low-grade inflammation has been associated with the presence of a worse cardiovascular profile (33) and the presence of micro- and macrovascular complications in subjects with type 1 diabetes (11). Prospective studies also have demonstrated the predictive value of low-grade inflammation in the development of chronic complications in this disease (34). Our study would be in agreement with these results, arterial stiffness being an early sign of arteriosclerosis.

aPWV predicts cardiovascular events and total and cardiovascular mortality in the general population, in the elderly, in patients with hypertension, in subjects with end-stage renal failure, and in subjects with type 2 diabetes (5). The independent predictive value of arterial stiffness has been demonstrated after adjustment for classical cardiovascular risk factors. This suggests that arterial stiffness measurement could add a value to the classical cardiovascular risk factors in the prediction of cardiovascular risk (35). This may be explained by the fact that arterial stiffness integrates the damage of cardiovascular risk factors (classical and nonclassical) on the aortic wall over a long period of time, whereas cardiovascular risk factors can fluctuate in time, and their values, recorded at the time of risk assessment, may not reflect their real impact in damaging the arterial wall (4). However, prospective studies are needed to establish the prognosis value of aPWV in subjects with type 1 diabetes regarding cardiovascular events. To the best of our knowledge, only one recent prospective study has evaluated the relationship between central arterial stiffness and the prediction of cardiovascular events in type 1 diabetes. This study showed that central PP was more strongly associated with the prediction of cardiovascular events than AIx, but neither data on aPWV nor markers of low-grade inflammation were reported (36).

**Table 4—Independent aPWV predictors for the whole population (by sex)**

	B (unstandardized)	SD	$\beta$ (standardized)	<i>P</i>
<b>Men</b>				
log aPWV ( <i>R</i> = 0.737; <i>R</i> <sup>2</sup> = 0.543)				
Constant	0.586	0.054	—	<0.001
Age	0.003	0.001	0.351	0.001
Low-grade inflammation score	0.028	0.011	0.287	0.019
BMI	0.005	0.002	0.225	0.024
Type 1 diabetes	0.030	0.014	0.246	0.035
<b>Women</b>				
log aPWV ( <i>R</i> = 0.741; <i>R</i> <sup>2</sup> = 0.550)				
Constant	0.377	0.085	—	<0.001
Age	0.004	0.001	0.509	<0.001
BMI	0.005	0.002	0.227	0.018
MAP	0.002	0.001	0.202	0.032
Type 1 diabetes	0.028	0.014	0.182	0.047

The following variables were adjusted for in this model: age, smoking, physical activity, hypertension (no/yes), dyslipidemia (no/yes), BMI, MAP, total cholesterol, log triglycerides, logHDL cholesterol, type 1 diabetes, and low-grade inflammation score. Only significant variables are shown in the table.

The major limitation of our study is its cross-sectional design, which makes it impossible to determine the temporal ordering of the association between arterial stiffness and increased levels of inflammatory-related serum proteins. In addition, its observational design does not allow us to ensure complete control of all the potential (unknown) confounding factors. The concentrations of the inflammatory-related serum proteins were measured only once, which might underestimate the association between them and arterial stiffness. Nevertheless, it should be noted that the low-grade inflammation general score was independently associated with aPWV in the multiple regression analyses.

In conclusion, our study demonstrates that aPWV is increased in subjects with type 1 diabetes compared with age- and sex-matched healthy subjects, even after controlling for classical cardiovascular risk factors. This suggests that the measurement of arterial stiffness could provide some additional information regarding cardiovascular risk in type 1 diabetes. Finally, our study shows, for the first time, that arterial stiffness is associated with an increase in inflammatory-related serum proteins in men with type 1 diabetes. Our findings suggest that arterial stiffness measurement is a useful tool for detecting subclinical arteriosclerosis and making a better cardiovascular prediction in type 1 diabetes. Additional studies exploring not only the link between arterial stiffness and low-grade inflammation but also its potential therapeutic implications are needed.

**Acknowledgments**—Financial support was provided by the Associació Catalana de Diabetis (Beca Gonçal Lloveras 2008); the Fundació la Marató de TV3-2008 (project no. 081410); FIS PS09/01360, Instituto de Salud Carlos III, Ministerio de Sanidad y Consumo, Spain; and by an intensification grant to J.M.G.-C. (Instituto de Salud Carlos III, Ministerio de Sanidad y Consumo, Spain).

No potential conflicts of interest relevant to this article were reported.

G.L. researched data, contributed to the discussion, and wrote, reviewed, and edited the manuscript. V.C.-M. and N.F. researched data. C.V., R.S., and J.V. contributed to the discussion and reviewed and edited the manuscript. J.M.G.-C. wrote, reviewed, and edited the manuscript and contributed to the discussion. J.M.G.-C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the

integrity of the data and the accuracy of the data analysis.

## References

- Daneman D. Type 1 diabetes. *Lancet* 2006; 367:847–858
- Libby P, Nathan DM, Abraham K, et al.; National Heart, Lung, and Blood Institute; National Institute of Diabetes and Digestive and Kidney Diseases Working Group on Cardiovascular Complications of Type 1 Diabetes Mellitus. Report of the National Heart, Lung, and Blood Institute-National Institute of Diabetes and Digestive and Kidney Diseases Working Group on Cardiovascular Complications of Type 1 Diabetes Mellitus. *Circulation* 2005;111:3489–3493
- Cavalcante JL, Lima JA, Redheuil A, Al-Mallah MH. Aortic stiffness: current understanding and future directions. *J Am Coll Cardiol* 2011;57:1511–1522
- Laurent S, Cockcroft J, Van Bortel L, et al.; European Network for Non-invasive Investigation of Large Arteries. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J* 2006;27:2588–2605
- Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J Am Coll Cardiol* 2010;55:1318–1327
- Kaptoge S, Di Angelantonio E, Lowe G, et al.; Emerging Risk Factors Collaboration. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet* 2010;375:132–140
- Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005;352:1685–1695
- Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* 2000;101:1767–1772
- Tuomisto K, Jousilahti P, Sundvall J, Pajunen P, Salomaa V. C-reactive protein, interleukin-6 and tumor necrosis factor alpha as predictors of incident coronary and cardiovascular events and total mortality: a population-based, prospective study. *Thromb Haemost* 2006;95:511–518
- Danesh J, Kaptoge S, Mann AG, et al. Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. *PLoS Med* 2008;5:e78
- Schram MT, Chaturvedi N, Schalkwijk CG, Fuller JH, Stehouwer CD; EURODIAB Prospective Complications Study Group. Markers of inflammation are cross-sectionally associated with microvascular complications and cardiovascular disease in type 1 diabetes: the EURODIAB Prospective Complications Study. *Diabetologia* 2005;48:370–378
- Nakhai-Pour HR, Grobbee DE, Bots ML, Muller M, van der Schouw YT. C-reactive protein and aortic stiffness and wave reflection in middle-aged and elderly men from the community. *J Hum Hypertens* 2007;21:949–955
- Mahmud A, Feely J. Arterial stiffness is related to systemic inflammation in essential hypertension. *Hypertension* 2005; 46:1118–1122
- Lilitkarntakul P, Dhaun N, Melville V, et al. Blood pressure and not uraemia is the major determinant of arterial stiffness and endothelial dysfunction in patients with chronic kidney disease and minimal co-morbidity. *Atherosclerosis* 2011;216:217–225
- Ferreira I, Boreham CA, Twisk JW, et al. Clustering of metabolic syndrome risk factors and arterial stiffness in young adults: the Northern Ireland Young Hearts Project. *J Hypertens* 2007;25:1009–1020
- González-Clemente JM, Giménez-Pérez G, Richart C, et al. The tumour necrosis factor (TNF)-alpha system is activated in accordance with pulse pressure in normotensive subjects with type 1 diabetes mellitus. *Eur J Endocrinol* 2005;153:687–691
- Arain FA, Kuniyoshi FH, Abdalrhim AD, Miller VM. Sex/gender medicine: the biological basis for personalized care in cardiovascular medicine. *Circ J* 2009;73:1774–1782
- Mancia G, De Backer G, Dominiczak A, et al.; The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension; The Task Force for the Management of Arterial Hypertension of the European Society of Cardiology. 2007 guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J* 2007;28:1462–1536
- National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002;106:3143–3421
- American Diabetes Association. Standards of medical care in diabetes: 2011. *Diabetes Care* 2011;34(Suppl. 1):S11–S61
- Lacy PS, O'Brien DG, Stanley AG, Dewar MM, Swales PP, Williams B. Increased

- pulse wave velocity is not associated with elevated augmentation index in patients with diabetes. *J Hypertens* 2004;22:1937–1944
22. Vastagh I, Horváth T, Nagy G, et al. Evolution and predictors of morphological and functional arterial changes in the course of type 1 diabetes mellitus. *Diabetes Metab Res Rev* 2010;26:646–655
  23. Schalkwijk CG, Poland DC, van Dijk W, et al. Plasma concentration of C-reactive protein is increased in type I diabetic patients without clinical macroangiopathy and correlates with markers of endothelial dysfunction: evidence for chronic inflammation. *Diabetologia* 1999;42:351–357
  24. Jenkins AJ, Rothen M, Klein RL, et al.; DCCT/EDIC Research Group. Cross-sectional associations of C-reactive protein with vascular risk factors and vascular complications in the DCCT/EDIC cohort. *J Diabetes Complications* 2008;22:153–163
  25. Kilpatrick ES, Keevil BG, Jagger C, Spooner RJ, Small M. Determinants of raised C-reactive protein concentration in type 1 diabetes. *QJM* 2000;93:231–236
  26. Mackness B, Hine D, McElduff P, Mackness M. High C-reactive protein and low paraoxonase 1 in diabetes as risk factors for coronary heart disease. *Atherosclerosis* 2006;186:396–401
  27. Haverkate F, Thompson SG, Pyke SD, Gallimore JR, Pepys MB; European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. Production of C-reactive protein and risk of coronary events in stable and unstable angina. *Lancet* 1997;349:462–466
  28. Lanza GA, Pitocco D, Navarese EP, et al. Association between cardiac autonomic dysfunction and inflammation in type 1 diabetic patients: effect of beta-blockade. *Eur Heart J* 2007;28:814–820
  29. Nowak M, Wielkoszyński T, Marek B, et al. Antioxidant potential, paraoxonase 1, ceruloplasmin activity and C-reactive protein concentration in diabetic retinopathy. *Clin Exp Med* 2010;10:185–192
  30. Tsioufis C, Dimitriadis K, Selima M, et al. Low-grade inflammation and hypoadiponectinaemia have an additive detrimental effect on aortic stiffness in essential hypertensive patients. *Eur Heart J* 2007;28:1162–1169
  31. Colhoun HM, Schalkwijk C, Rubens MB, Stehouwer CD. C-reactive protein in type 1 diabetes and its relationship to coronary artery calcification. *Diabetes Care* 2002;25:1813–1817
  32. Zieman SJ, Melenovsky V, Kass DA. Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol* 2005;25:932–943
  33. Schram MT, Chaturvedi N, Schalkwijk C, et al.; EURODIAB Prospective Complications Study. Vascular risk factors and markers of endothelial function as determinants of inflammatory markers in type 1 diabetes: the EURODIAB Prospective Complications Study. *Diabetes Care* 2003;26:2165–2173
  34. Astrup AS, Tarnow L, Pietraszek L, et al. Markers of endothelial dysfunction and inflammation in type 1 diabetic patients with or without diabetic nephropathy followed for 10 years: association with mortality and decline of glomerular filtration rate. *Diabetes Care* 2008;31:1170–1176
  35. Boutouyrie P, Tropeano AI, Asmar R, et al. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. *Hypertension* 2002;39:10–15
  36. Gordin D, Wadén J, Forsblom C, et al.; FinnDiane Study Group. Pulse pressure predicts incident cardiovascular disease but not diabetic nephropathy in patients with type 1 diabetes (The FinnDiane Study). *Diabetes Care* 2011;34:886–891