



Sickle Cell Trait Worsens Oxidative Stress, Abnormal Blood Rheology, and Vascular Dysfunction in Type 2 Diabetes

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OBJECTIVE

It is predicted that Africa will have the greatest increase in the number of patients with type 2 diabetes mellitus (T2DM) within the next decade. T2DM patients are at risk for cardiovascular disorders. In Sub-Saharan African countries, sickle cell trait (SCT) is frequent. Despite the presence of modest abnormalities in hemorheology and oxidative stress, SCT is generally considered a benign condition. Little is known about vascular function in SCT, although recent studies demonstrated an increased risk of cardiovascular disorders, including venous thromboembolism, stroke, and chronic kidney disease. We hypothesized that SCT could accentuate the vascular dysfunction observed in T2DM.

RESEARCH DESIGN AND METHODS

The current study, conducted in Senegal, compared vascular function, hemorheological profile, and biomarkers of oxidative stress, inflammation, and nitric oxide metabolism in healthy individuals (CONT), subjects with T2DM or SCT, and patients with both T2DM and SCT (T2DM-SCT).

RESULTS

Flow-mediated dilation was blunted in individuals with T2DM, SCT, and T2DM-SCT compared with CONT, with vascular dysfunction being most pronounced in the latter group. Carotid-femoral pulse wave velocity measurements demonstrated increased arterial stiffness in T2DM-SCT. Oxidative stress, advanced glycation end products, and inflammation (interleukin-1 β) were greater in patients with T2DM-SCT compared with the other groups. Blood viscosity was higher in individuals with T2DM, SCT carriers, and individuals with T2DM-SCT, and the values were further increased in the latter group.

CONCLUSIONS

Our results demonstrate severe biological abnormalities and marked vascular dysfunction in patients with both T2DM and SCT. SCT should be viewed as a risk factor for further cardiovascular disorders in individuals with T2DM.

Chronic hyperglycemia, hyperlipidemia, and hyperinsulinemia cause endothelial and vascular dysfunction in large arteries and microcirculation of patients with type 2 diabetes mellitus (T2DM) (1). Elevated glycemia accelerates the formation of advanced glycation end products (AGEs), which generate reactive oxygen species (ROS) and promote inflammation (2). Impaired endothelial nitric oxide (NO)

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synthase activity due to progressive insulin resistance and reduced NO bioavailability caused by enhanced oxidative stress result in impaired vascular reactivity (1). T2DM is a major public health problem worldwide, and recent epidemiological data predict that Africa, and more particularly Sub-Saharan countries, will have the greatest increase in the number of people with T2DM, from 19.8 million in 2013 to 41.4 million in 2035, if current trends persist (3).

Concomitantly, in Sub-Saharan African countries, sickle cell trait (SCT) is highly prevalent (4). SCT is the heterozygous form of sickle cell anemia, a severe disease resulting from a single genetic mutation occurring on the β -globin gene and responsible for the synthesis of abnormal hemoglobin (Hb), known as HbS. HbS has a propensity to polymerize under deoxygenated conditions, resulting in sickling of red blood cells. Whereas patients with sickle cell anemia are frequently exposed to severe acute/chronic complications, SCT carriers are usually asymptomatic because the presence of normal Hb (HbA) in red blood cells limits HbS polymerization (5). However, very recent epidemiological data on large U.S. cohorts demonstrated that SCT increases the risks for venous thromboembolism (6,7), ischemic stroke (8), and glomerulopathy (9) in Afro-American individuals. Although never investigated, these findings may suggest that vascular function could be impaired in SCT (10). The high prevalence of SCT in Sub-Saharan Africa and the growing proportion of this population with T2DM imply that a high number of individuals may have both conditions. Although the association of T2DM and SCT has been suspected to increase the risks for vascular disorders (11), no study has investigated vascular function in this population. We hypothesized that SCT could accentuate the vascular dysfunction observed in T2DM.

The current study, conducted in Senegal, compared for the first time the vascular function, hemorheological profile, and biomarkers of oxidative stress, inflammation, NO metabolism, and vascular adhesion process between healthy individuals, subjects with T2DM, SCT carriers, and patients with both T2DM and SCT.

RESEARCH DESIGN AND METHODS

Participants

The current study was conducted at the Cheikh Anta Diop University (UCAD). The protocol was designed in accordance

with the Declaration of Helsinki and was approved by the Ethics Committee of Cheikh Anta Diop University (008/2013/CER/UCAD). Participants were informed of the procedures and purposes of the study and gave written informed consent to participate. Twenty-eight individuals with T2DM were randomly selected at the Anti-diabetes Centre (Marc Sanakalé) at the Abass Ndao Hospital in Dakar: 14 without and 14 with SCT (groups with T2DM and T2DM-SCT, respectively). Thirty-two individuals without diabetes or any other known diseases were recruited in the general population of Dakar and in blood donors from the National Center of Blood Transfusion (CNTS) of Dakar: 14 without SCT (CONT) and 18 individuals with SCT (SCT). The presence or absence of SCT in each individual was already known by the CNTS or the Anti-diabetes Centre and was confirmed by biological analysis (isoelectric focusing, citrate agar electrophoresis, high-performance liquid chromatography, and a solubility test). Patients with leg ulcers or cardiac disorders were excluded from the study. All participants were Senegalese.

Blood Samples

All the subjects arrived at the Laboratory of Medical Physiology (Cheikh Anta Diop University) at 8:00 A.M. in fasting condition. They were asked to refrain from any physical activity for at least 24 h before the experimental day. Blood was drawn into fluoride tubes (5 mL) for glucose measurement, heparin tubes (5 mL) for lipid measurement, citrate tubes (5 mL) for plasma fibrinogen determination, and EDTA tubes (5 mL) for the analyses of blood rheology, hemoglobin A_{1c} (HbA_{1c}), oxidative stress, and inflammatory and NO metabolism biomarkers.

Routine Biochemical Parameters

Plasma lipids (triglycerides [TG] and total, HDL, and LDL cholesterol), fibrinogen, and glucose levels were evaluated using standard enzymatic methods. HbA_{1c} was measured using high-performance liquid chromatography (DiaStat; Bio-Rad, Hercules, CA).

Hemorheological Parameters

Blood viscosity was measured with a cone-plate viscometer (Pro DV-II+, with CPE40 spindle; Brookfield, Middleboro, MA) at varying shear rates (5.62, 11.25,

22.5, 45, 90, and 225 s⁻¹) and 37°C, as recommended (12). Plasma viscosity was evaluated at 750 s⁻¹ and 37°C. Hematocrit (Hct) was measured after blood microcentrifugation (1,500g, 5 min, 25°C; Jouan-Hema-C, Saint Herblain, France).

Markers of Oxidative Stress, NO Metabolism, and Inflammation

Blood was rapidly centrifuged after sampling and plasma was stored at -80°C until analysis.

Advanced Oxidation Protein Products

Advanced oxidation protein products (AOPPs) are plasma compounds preferentially formed from albumin by chlorinated oxidants produced by myeloperoxidase. AOPPs stimulate both monocyte respiratory burst and TNF- α synthesis (13), which participates in the pathophysiology of several cardiovascular disorders associated with phagocyte-derived oxidative stress (14). AOPPs were determined in plasma using a semiautomated method (15) based on the absorption of AOPPs at 340 nm and at low pH. AOPPs were measured by spectrophotometry after addition of acetic acid and were calibrated with a chloramine-T solution that absorbs at 340 nm in the presence of potassium iodide. The absorbance of the plasma diluted in PBS (1:4, volume for volume) mixed with acetic acid was immediately read at 340 nm against a blank containing PBS, potassium iodide, and acetic acid.

Malondialdehyde

Concentrations of plasma malondialdehyde (MDA) were determined as thiobarbituric-reactive substances (16). The pink chromogen, resulting from the fixation of thiobarbituric acid to MDA at low pH and after 1 h at 100°C, was extracted with *n*-butanol and its absorbance was measured at 532 nm by spectrophotometry using 1,1,3,3-tetraethoxypropan as standard.

AGEs

AGEs in plasma were determined by ELISA according to the manufacturer's instructions (Cell Biolabs, San Diego, CA).

Nitrotyrosine

Concentration of total (free + protein bound) plasma nitrotyrosine, as an end product of protein nitration by peroxynitrite (ONOO⁻), was measured by a competitive ELISA test using 96-well plates coated with nitrated BSA at 5 μ g/mL in coating buffer (50 mmol/L NaHCO₃), as previously described (17).

Ferric-Reducing Antioxidant Power

Ferric-reducing antioxidant power (FRAP) was measured according to previous method (18) based on the ferric-reducing ability of plasma. The absorbance of the plasma in the presence of Fe^{3+} and tripyridyltriazine was read after 4 min at 593 nm using an aqueous solution of known Fe^{2+} concentration ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) as standard.

NO End Products

NO end products (NOx) (sum of nitrite and nitrate) were measured in plasma using the Griess method previously described (19). Plasma was previously incubated with nitrate (NO_3^-) reductase and nicotinamide adenine dinucleotide phosphate for 40 min to reduce NO_3^- in NO_2^- .

Soluble Vascular Cell Adhesion Molecule-1 and Interleukin-1 β

Soluble vascular cell adhesion molecule-1 (sVCAM-1) and interleukin-1 β (IL-1 β) were assayed by ELISA according to the manufacturer's instructions (Diacclone Systems, Besançon, France).

Limitations

The possible interference of color components in plasma on the absorbance measured for AOPP, MDA, FRAP, and NOx assays is a limitation of these techniques.

Flow-Mediated Dilatation

Flow-mediated dilatation (FMD) of the brachial artery was measured by the same experienced cardiologist and by ultrasound according to the guidelines described by Corretti et al. (20). Brachial artery ultrasonography was performed after resting in the supine position for 15 min and in the fasting state. All participants refrained from drinking beverages containing caffeine or alcohol for 12 h before the examination and were also advised not to take antihypertensive or vasodilator drugs the day of examination. Patients were examined in a quiet and temperature-controlled room (25°C). The right arm was extended and immobilized with an angle of $\sim 60^\circ$ from the trunk of the body. A 10-MHz linear transducer connected to an ultrasound device (Sonoline G50; Siemens) was placed on the brachial artery at 1–2 cm proximal to the elbow joint. After scanning the baseline artery diameter, the cuff was rapidly inflated to 50 mmHg above systolic blood pressure and kept for 5 min. By rapid deflation of the cuff, reactive hyperemia was induced and scanning was performed at 5, 30, 60,

90, and 120 s and 10 min after cuff deflation to obtain the FMD, expressed in percentage of the baseline diameter (%FMD). Because of limited technical (software) resources, we were not able to capture the diameter continuously. FMD was measured in duplicate for each patient with at least 1 h in resting condition between the two measurements, and the mean of the two FMD values was calculated. A difference of $<10\%$ between the two measurements was considered as acceptable. The cardiologist who performed FMD and pulse wave velocity (PWV) experiments was blinded to the diagnosis of the patient.

Blood Velocity, Shear Rate, and Shear Stress

The blood velocity profile was obtained at the same time as diameter determination using a 7.5–10-MHz linear transducer B mode operated in the high-pulsed repetition frequency mode (2–25 kHz) with a sample volume of 1.5–3.5 cm in depth. Care was taken to avoid bias by using scale adjustments, especially after cuff release. In duplex mode, real-time ultrasound imaging and PWV profile were viewed simultaneously during the cardiac cycle (systolic and diastolic), and shear rate was calculated using the following equation: $4 \times \text{mean blood velocity (cm/s)} / \text{diameter (cm)}$ (21). Shear stress was the product of blood viscosity by shear rate (22). Most of the previous studies in the field of cardiovascular physiology used a single and/or unmeasured blood viscosity (i.e., estimated from Hct level) value to calculate shear stress, which introduced biased values. Blood is a shear-thinning fluid, with its viscosity value being affected by the shear rate (23). Indeed, we used the blood viscosity values obtained with the cone plate viscometer to calculate the individualized shear stress for every participant. Blood viscosity level was chosen at the viscometer's shear rate, corresponding to the in vivo shear rate calculated from blood velocity and diameter.

Systolic and Diastolic Arterial Pressure and PWV

Systolic and diastolic blood pressures were manually measured three times in the left arm using a manual sphygmomanometer (Omron M3; Intellisense, Kyoto, Japan) in standardized sitting position and after 30 min of rest (24). The carotid-femoral PWV was measured with an

automated system (Pulse Pen; DiaTecne, Milan, Italy). The carotid and femoral waveforms were acquired simultaneously with two pressure-sensitive transducers, and the transit time of the pulse was calculated by the system software. The distance between the two arterial sites was measured on the body using a tape measure, and the PWVs were calculated as the distance divided by time (m/s). At least 12 successive readings were used for analysis to cover a complete respiratory cycle. All PWV measurements were performed by the same operator, and the mean of three consecutive measures (each taken 5 min apart) was calculated.

Associated Complications

Three complications were investigated: retinopathy by indirect ophthalmoscopy with a noncontact slip lamp lens (25), hypertension using the American Heart Association recommendation (24), and albuminuria by using the Hemocue 20 system (Angelholm, Sweden) (26).

Statistical Analysis

Results are expressed as mean \pm SD. Anthropometric, biochemical, hemodynamic, and hemorheological parameters; oxidative stress biomarkers; and adhesion molecules were compared between the four groups using a one-way ANOVA with Holm-Bonferroni correction. χ^2 or Fischer exact test was used to compare the frequency of retinopathy, hypertension, and glomerulopathy between the groups. Pearson correlation was used to test the associations between FMD and several biomarkers by pooling the groups. Multivariate linear regression model was used to predict the independent associations between different variables of interest with vascular dysfunction. The significance level was defined as $P < 0.05$. Analyses were conducted using SPSS software (version 20; IBM SPSS Statistics, Chicago, IL).

RESULTS

Anthropometrics, Blood Pressure, Biochemical Parameters, and Associated Complications

None of the participants included in this study had a previous history of smoking. The percentage of patients under metformin (Glucophage) treatment was not significantly different between the groups with T2DM-SCT (36%) and T2DM (50%). Two patients of the group with T2DM-SCT were treated with

calcium channel blocker (Amlor) and one of the group with T2DM with ACE inhibitor (Trizatide). Sex distribution was not significantly different between the four groups. The duration of diabetes was not significantly different between the groups with T2DM-SCT (2.9 ± 1.7 years) and T2DM (3.0 ± 1.9 years).

BMI was greater in patients with T2DM compared with subjects without diabetes independently of SCT (Table 1). The group with T2DM was older than the three other groups (Table 1). Whereas no significant difference in diastolic blood pressure was found between the four groups after Holm-Bonferroni correction, we found significant differences regarding systolic blood pressure: higher in patients with T2DM-SCT and patients with T2DM compared with individuals with SCT and higher in the group with T2DM than in CONT subjects (Table 1). Fasting glucose and HbA_{1c} levels were higher, and HDL concentration was lower, in patients with diabetes independently of SCT, compared with the subjects without diabetes. Fibrinogen was greater in individuals with T2DM-SCT compared with the CONT group and group with T2DM. TG level was not different between the four groups. A higher frequency of participants with both T2DM and SCT had arterial hypertension (64.3 vs. 14.3, 16.7, and 50.0% in the CONT group, group with SCT, and group with T2DM, respectively; $P < 0.01$) or microalbuminuria (57.1 vs. 0,

0, and 28.6% in the CONT group, group with SCT, and group with T2DM, respectively; $P < 0.001$). Retinopathy was more frequent in the two groups with diabetes (42.9 and 50% in the groups with T2DM and T2DM-SCT, respectively) compared with the two groups without diabetes (0 and 5.6% in the CONT group and group with SCT, respectively; $P < 0.01$).

Hemorheological Parameters

Hct was not different between the four groups (Table 2). Blood viscosity was higher in the groups with T2DM-SCT, T2DM, and SCT compared with the CONT group at all shear rates. The two groups with diabetes also had higher blood viscosity than the group with SCT in the $5.52\text{--}45\text{ s}^{-1}$ shear rate range. At 90 s^{-1} , blood viscosity tended to be higher in patients with T2DM-SCT compared with patients with T2DM ($P = 0.066$) and individuals with SCT ($P = 0.064$). At 225 s^{-1} , blood viscosity was significantly different between the groups with T2DM-SCT and T2DM. Plasma viscosity of patients with diabetes (with or without SCT) was higher than in subjects without diabetes in the two other groups.

Markers of Oxidative Stress, Inflammation, and NO Metabolism Parameters

The two groups with diabetes had higher AGEs and IL-1 β levels than individuals with SCT and CONT individuals but, most importantly, AGEs and IL-1 β

concentrations were higher in the group with T2DM-SCT than in patients with T2DM (Table 3). AOPP was greater in the group with T2DM-SCT and tended to be higher in individuals with SCT ($P = 0.072$) compared with the CONT group. Nitrotyrosine level was greater in patients with diabetes and SCT than in the group with SCT and the CONT group. T2DM-SCT was the only group expressing higher MDA concentrations compared with the CONT group. No significant difference was observed for FRAP, VCAM-1, and NOx between the four groups.

Baseline Hemodynamic Parameters, FMD, and PWV

Diastolic and systolic diameters were not different between the four groups (Table 2). Whereas systolic blood flow did not differ between the four groups, diastolic blood flow was increased in the groups with T2DM-SCT and T2DM compared with the two other groups. Systolic shear rate was not different between the four groups, whereas diastolic shear rate was higher in the two groups with diabetes compared with the CONT group (Table 2). Diastolic shear rate was also higher in T2DM-SCT than in individuals with SCT. Systolic shear stress of patients with T2DM-SCT was increased in comparison with CONT. Diastolic shear stress was increased in the two groups with T2DM compared with CONT individuals. Moreover, the group with T2DM-SCT expressed higher diastolic shear stress than the group with SCT. Despite these differences in shear rates and shear stresses, baseline arterial diameter was not different between the four groups.

Figure 1 shows the kinetics of FMD after cuff deflation in the four groups. Significant differences were observed at 60, 90, and 120 s. When looking at the details for the FMD determined at 90 s after cuff deflation (Fig. 2A), patients with T2DM-SCT expressed lower FMD than the three other groups. We also noted a reduced FMD in patients with T2DM compared with the CONT group and a trend for SCT carriers to have lower FMD than healthy controls ($P = 0.066$). ANCOVA with age, sex, BMI, and arterial pressures as covariates also demonstrated lower FMD in the patients with T2DM-SCT ($P < 0.01$) compared with the other groups. FMD measured 90 s after cuff deflation correlated with AGEs ($P < 0.05$; $r = -0.28$), MDA ($P < 0.05$; $r = -0.36$), sVCAM-1 ($P < 0.05$; $r = -0.30$),

Table 1—Characteristics and biochemical parameters of patients with diabetes with (T2DM-SCT) or without (T2DM) SCT and of healthy individuals with (SCT) or without (CONT) SCT

	T2DM-SCT	T2DM	SCT	CONT
Sex (male/female)	7/7	7/7	8/10	8/6
Age (years)	42.4 ± 9.3	$52.8 \pm 11.6^{***,†,‡}$	42.8 ± 8.2	40.4 ± 5.7
BMI (kg/m^2)	$29.5 \pm 4.7^{**},+++$	$27.0 \pm 4.6^{*,†}$	23.6 ± 3.6	24.4 ± 2.8
Diastolic BP (mmHg)	84 ± 10	86 ± 10	72 ± 10	77 ± 10
Systolic BP (mmHg)	$128 \pm 17^{\dagger}$	$131 \pm 16^{*,+++}$	118 ± 14	119 ± 13
HbS (%)	36.0 ± 2.0	—	38.9 ± 2.7	—
Glucose (g/L)	$1.7 \pm 0.6^{***},+++$	$1.8 \pm 0.6^{***},+++$	1.0 ± 0.1	0.9 ± 0.1
HbA _{1c} (%)	$7.5 \pm 3.0^{**},++$	$7.3 \pm 2.0^{*,++}$	4.8 ± 1.2	5.0 ± 1.2
(mmol/mol)	$58 \pm 9^{**},++$	$56 \pm 0^{*,++}$	29 ± 0	31 ± 0
LDL (g/L)	$1.1 \pm 0.4^{\dagger}$	1.0 ± 0.3	0.7 ± 0.4	0.7 ± 0.3
HDL (g/L)	$0.5 \pm 0.2^{***},++$	$0.6 \pm 0.3^{**},\dagger$	0.8 ± 0.2	0.9 ± 0.2
TG (g/L)	1.5 ± 0.6	1.2 ± 0.7	1.2 ± 0.3	1.3 ± 0.4
T-Chol (g/L)	1.8 ± 0.4	1.7 ± 0.3	1.5 ± 0.5	1.4 ± 0.4
Fibrinogen (g/L)	$5.3 \pm 1.7^{*},\#$	3.9 ± 1.9	4.0 ± 1.0	3.4 ± 1.0

Data are means \pm SD. BP, blood pressure; T-Chol, total cholesterol. *Different from CONT, $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. \dagger Different from SCT, $P < 0.05$. $++P < 0.01$. $+++P < 0.001$. #Different from T2DM, $P < 0.05$. \ddagger Different from T2DM-SCT, $P < 0.05$.

Table 2—Hemorheological and hemodynamic parameters of patients with diabetes with (T2DM-SCT) or without (T2DM) SCT and of healthy individuals with (SCT) or without (CONT) SCT

	T2DM-SCT	T2DM	SCT	CONT
Hct (%)	40.6 ± 2.3	41.3 ± 2.1	41.8 ± 2.2	40.5 ± 1.3
Blood viscosity (cP)				
5.62 s ⁻¹	11.1 ± 1.0***,†	10.9 ± 0.8***,†	10.2 ± 0.8**	9.2 ± 0.9
11.25 s ⁻¹	9.7 ± 1.0***,†	9.8 ± 0.8***,††	8.9 ± 0.6*	8.2 ± 0.8
22.5 s ⁻¹	8.7 ± 0.9***,††	8.7 ± 0.6***,†††	7.9 ± 0.5*	7.2 ± 0.6
45 s ⁻¹	7.7 ± 0.8***,†	7.6 ± 0.5***,†	7.1 ± 0.5*	6.4 ± 0.3
90 s ⁻¹	7.0 ± 0.7***	6.5 ± 0.4*	6.5 ± 0.5***	5.8 ± 0.4
225 s ⁻¹	6.3 ± 0.7***,#	5.8 ± 0.4***	5.9 ± 0.4***	4.9 ± 0.4
Plasma viscosity (cP)	1.8 ± 0.4**,††	1.8 ± 0.4**,††	1.3 ± 0.3	1.4 ± 0.3
Baseline diameter (mm)				
Systolic	4.0 ± 0.5	4.0 ± 0.5	4.0 ± 0.4	4.2 ± 0.5
Diastolic	3.7 ± 0.4	3.8 ± 0.5	3.8 ± 0.4	3.9 ± 0.5
Baseline blood flow (mL/min)				
Systolic	331 ± 112	310 ± 120	299 ± 98	292 ± 88
Diastolic	286 ± 106**,††	266 ± 84*,†	202 ± 40	197 ± 53
Baseline shear rate (s ⁻¹)				
Systolic	504.5 ± 135.5	452.0 ± 117.0	445.0 ± 120.1	386.0 ± 99.8
Diastolic	156.8 ± 63.3***,†	142.1 ± 47.8*	110.3 ± 31.2	95.7 ± 25.4
Baseline shear stress (dyn/cm ²)				
Systolic	26.7 ± 9.5***	22.3 ± 6.4	23.2 ± 7.1	16.6 ± 3.9
Diastolic	10.5 ± 4.5***,††	8.9 ± 2.8*	7.1 ± 2.0	5.6 ± 1.6

Data are means ± SD. *Different from CONT, $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. †Different from SCT, $P < 0.05$. †† $P < 0.01$. ††† $P < 0.001$. #Different from T2DM, $P < 0.05$.

IL-1 β ($P < 0.05$; $r = -0.37$), and NOx ($P < 0.05$; $r = 0.28$). Unfortunately, it was not possible to normalize FMD values by nitroglycerin-mediated dilation, which represents the maximal dilation that can be achieved. We were able to correct the FMD values at 90 s after cuff deflation by the shear stress measured at that time (corrected FMD). We still observed lower corrected FMD in the group with T2DM-SCT compared with subjects with T2DM, subjects with SCT, and CONT subjects (Fig. 2B). The group with T2DM and the group with SCT had lower corrected FMD than the CONT group, and SCT carriers and patients with T2DM were significantly different. Mean FMD was decreased in individuals with T2DM-SCT compared with the CONT group (Fig. 2C). Corrected

FMD measured at 90 s correlated with FRAP ($P < 0.05$; $r = 0.35$), AGEs ($P < 0.001$; $r = -0.42$), nitrotyrosine ($P < 0.05$; $r = -0.32$), MDA ($P < 0.05$; $r = -0.33$), sVCAM-1 ($P < 0.05$; $r = -0.26$), IL-1 β ($P < 0.001$; $r = -0.52$), and AOPP ($P < 0.05$; $r = -0.25$). Carotid-femoral PWV was increased in the group with T2DM-SCT compared with the three other groups and in patients with T2DM compared with the CONT group (Fig. 2D). PWV correlated with AOPP ($P < 0.05$; $r = 0.32$), IL-1 β ($P < 0.01$; $r = 0.38$), and AGEs ($P < 0.01$; $r = 0.42$).

Multivariate Analyses

Because of the limited sample size ($n = 60$), only seven variables were included in the model to predict FMD measured

at 90 s: sex, BMI, age, LDL, nitrotyrosine, AGEs, and IL-1 β levels. Systolic and diastolic blood pressures, as well as lipids markers (except for LDL), were not included in the model because of the high risk of colinearity effect (variance inflation factor >3). The other lipid characteristics were not included in the model because of the high risk of colinearity with BMI. The overall model was statistically significant ($R^2 = 0.23$; $df = 7$; $P < 0.05$), and IL-1 β was the only factor independently associated with FMD ($\beta = -0.35$; $P = 0.034$). When replacing FMD with the corrected FMD, the overall model was still significant ($R^2 = 0.38$; $df = 7$; $P < 0.01$) and IL-1 β remained the only factor independently

Table 3—Markers of oxidative stress, inflammation, and NO metabolites of patients with diabetes with (T2DM-SCT) or without (T2DM) SCT and of healthy individuals with (SCT) or without (CONT) SCT

	T2DM-SCT	T2DM	SCT	CONT
AOPP ($\mu\text{mol/L}$)	122.9 ± 47.1**	88.1 ± 32.6	93.6 ± 66.0	63.1 ± 20.3
MDA ($\mu\text{mol/L}$)	37.3 ± 16.7*	30.3 ± 16.0	26.8 ± 7.1	22.9 ± 11.5
Nitrotyrosine ($\mu\text{mol/L}$)	1.8 ± 1.2***,†	1.1 ± 0.6	0.8 ± 0.5	0.6 ± 0.5
AGEs ($\mu\text{g/mL}$)	2.7 ± 1.0***,†††,###	1.5 ± 0.9*,†	0.7 ± 0.8	0.5 ± 0.4
FRAP ($\mu\text{mol/L}$)	449.2 ± 144.4	555.3 ± 127.6	568.5 ± 243.3	627.5 ± 269.0
sVCAM-1 (ng/mL)	1,079.4 ± 657.2	826.2 ± 179.1	897.1 ± 309.3	724.0 ± 178.8
IL-1 β (ng/mL)	7.2 ± 3.0***,†††,###	3.8 ± 2.1*,†	1.9 ± 1.0	1.7 ± 0.9
NOx ($\mu\text{mol/L}$)	17.8 ± 5.9	21.2 ± 11.0	23.4 ± 9.5	21.9 ± 4.4

Data are means ± SD. *Different from CONT, $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. †Different from SCT, $P < 0.05$. ††† $P < 0.001$. ###Different from T2DM, $P < 0.001$.

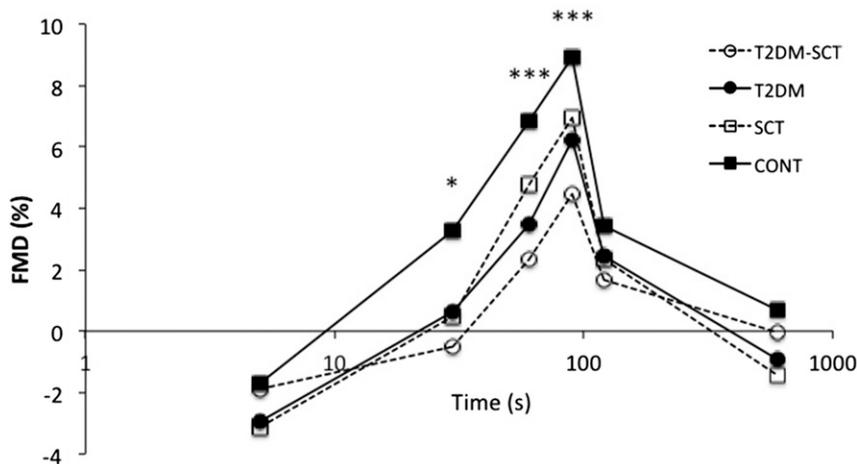


Figure 1—Kinetics (log scale) of mean FMD after cuff deflation in patients with diabetes with (T2DM-SCT) or without (T2DM) SCT and of healthy individuals with (SCT) or without (CONT) SCT. SD values are not shown to avoid a cluttered figure. Significant differences between groups: * $P < 0.05$; *** $P < 0.001$.

CONCLUSIONS

Our study is the first to suggest that SCT could worsen the vascular dysfunction among patients with T2DM. In addition, we found elevated blood viscosity and oxidative stress in patients with T2DM-SCT, which, in association with the vascular dysfunction, could increase the risk for vascular disorders in this population.

The principal aim of this study was to test whether the presence of SCT could worsen the vascular dysfunction and the biological abnormalities observed in patients with T2DM. Despite the fact that individuals with T2DM were older than subjects with T2DM-SCT, their vascular function was less altered. PWV, FMD, and corrected FMD were blunted in subjects with T2DM-SCT compared with the three other groups, demonstrating increased arterial stiffness and a loss of vascular reactivity in these subjects, which, in other diseases, have been shown to increase the risks for

associated with corrected FMD ($\beta = -0.32$; $P = 0.047$). When replacing FMD with the PWV, the model was also significant ($R^2 = 0.31$; $df = 7$; $P < 0.05$)

and AGEs was independently associated with PWV ($\beta = 0.40$, $P = 0.018$). Replacement of nitrotyrosine with AOPP in the models did not affect the results.

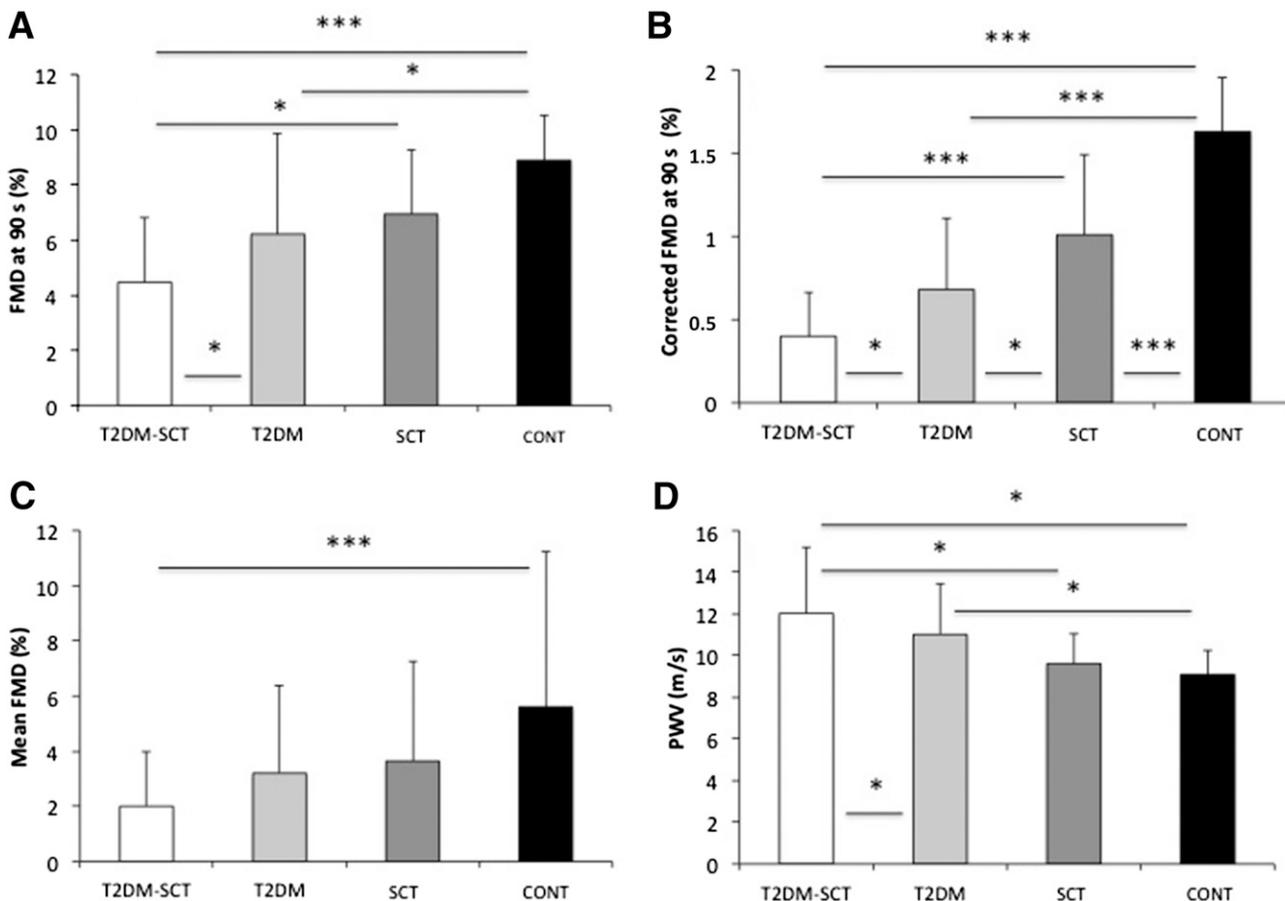


Figure 2—FMD and PWV of patients with diabetes with (T2DM-SCT) or without (T2DM) SCT and of healthy individuals with (SCT) or without (CONT) SCT. FMD measured at 90 s (A), FMD measured at 90 s corrected by shear stress values (B), mean FMD determined over 10 min after cuff deflation (C), and PWV (D) in the different groups. The two extremities of the horizontal bars indicate the difference between two given groups (* $P < 0.05$; *** $P < 0.001$). Means \pm SD.

cardiovascular morbidity and/or mortality (27,28). In agreement with this assumption, a higher number of participants with both T2DM and SCT had arterial hypertension or microalbuminuria compared with the three other groups. However, these findings need to be confirmed in larger cohorts. Moreover, we do not know how many females of the current study have achieved menopause, nor the effect of menopause on vascular function and cardiovascular morbidities in females with both T2DM and SCT.

Although the degree of metabolic abnormalities (i.e., lipids and glucose levels) was similar in the groups with T2DM and T2DM-SCT, the levels of plasma AOPP and MDA were increased in T2DM-SCT, suggesting enhanced ROS production in this population. We suspect that the slightly increased oxidative stress related to SCT, which has previously been reported in this population (29), could have promoted the greater AGE formation and nitrotyrosine observed in the group with T2DM-SCT (30). The inducible NO synthase (iNOS) activity is usually upregulated in the context of inflammation (31), which would result in higher NOx levels. However, the enhanced oxidative stress found in the group with T2DM-SCT could have led to the rapid formation of peroxynitrite (ONOO^-) (32), which could also explain the absence of difference in plasma NOx levels between the groups. The ligation of AGE to its receptor (RAGE) on endothelial cells may promote oxidative stress, inflammation, and endothelial activation (33). The negative correlations found between FMD, corrected FMD, or PWV and the different markers of oxidative stress and inflammation suggest a role of these biological abnormalities in the impairment of vascular function. The pathological role of oxidative stress in vascular dysfunction in diabetes is well known. Acute vitamin C infusion has been shown to improve FMD (34) and to limit the FMD impairment in response to hyperglycemia in patients with diabetes (35). Further experiments are needed to directly demonstrate the role of oxidative stress in vascular dysfunction in subjects with T2DM-SCT.

Multivariate analyses suggest that inflammation and AGE could be the most important factors playing a role in the vascular dysfunction and arterial remodeling,

respectively, since IL-1 β and AGE were independently associated with the FMD and PWV values, respectively. Growing evidence in the literature suggests a key role of inflammation in triggering vascular dysfunction in various diseases (36,37). For instance, mild to moderate inflammation may decrease the transcription of the endothelial NOS, which would result in lower NOx levels. At the same time, inflammation may upregulate iNOS. However, inflammation also promotes the production of ROS, which, by reacting with NO, would lead to the formation of peroxynitrite. In addition, this accumulation of free radicals may catalyze the transformation of arachidonic acid to F2-isoprostanes, such as 8-epi PDF2 α , a potent vasoconstrictor. Finally, inflammation may stimulate the production of cyclo-oxygenase-derived prostanoids, which would cause vasoconstriction. Although the cause-effect relationship between enhanced inflammation and vascular dysfunction in patients with T2DM-SCT is not established in our study, one may suspect that anti-inflammatory medication could be beneficial in this population. Further studies are needed to test this hypothesis.

Blood viscosity measured at high shear rate (i.e., at 225 s^{-1}) was higher in patients with T2DM-SCT than in patients with T2DM. Because plasma viscosity and Hct were not different between these two groups, one may suggest that the greater blood viscosity found in the former group was related to a reduction in red blood cell deformability (23,38,39). Both reduced red blood cell deformability and increased blood viscosity increase the risk for micro- and macrovascular complications (23). Indeed, as patients with T2DM-SCT display higher blood viscosity and fibrinogen than patients with only T2DM, they could be at higher risk for developing cardiovascular complications than the other groups, including patients with T2DM.

Our study suggests that SCT exacerbates inflammation and oxidative stress in patients with T2DM, leading to further impairment of vascular function and blood rheology, which could in turn increase the risk for developing micro- and macrovascular disorders. Although larger cohort studies are needed to explore the link between these diseases/genetic conditions and the development of several cardiovascular complications, we

suggest that patients with both T2DM and SCT should be more frequently monitored for cardiovascular health status. Further studies are also needed to test the effects of oxidative stress inhibition/modulation on the vascular function of individuals with T2DM-SCT. Based on the predictions of T2DM prevalence within the next 10 years in African regions where the occurrence of SCT can be 10–15% or greater, this detrimental association could become a worldwide health issue.

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Author Contributions. M.D. and P.C. designed the research, performed experiments, analyzed results, made the figures, interpreted data, and wrote the paper. V.P. and C.M. performed experiments, analyzed results, made the figures, interpreted data, and wrote the paper. A.S. wrote the paper. S.D., C.F., P.M., N.S.D., S.-N.D., and M.N.M. performed experiments and wrote the paper. B.R. analyzed results, made the figures, and wrote the paper. M.N.M. performed experiments and wrote the paper. N.S.K. interpreted data and wrote the paper. P.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.

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