Increased Shear Rate Resistance and Fastest Kinetics of Erythrocyte Aggregation in Diabetes Measured With Ultrasound

GUY CLOUTIER, PHD[1,2]
AUDREY ZIMMER, MSC[1]
FRANÇOIS T.H. YU, MSC[1]
JEAN-LOUIS CHIASSON, MD[3]

OBJECTIVE — To measure with ultrasound the increased erythrocyte aggregation (EA) kinetics and adhesion energy between erythrocytes in patients with type 2 diabetes and poor metabolic control.

RESEARCH DESIGN AND METHODS — Blood samples were analyzed in a Couette rheometer at 32 MHz following shear rate reductions from 500 s^{-1} to residual shears of 0 (stasis), 1, 2, 10, 50, 100, and 200 s^{-1}. The increase in EA was determined with the integrated backscatter coefficient as a function of time and shear rate.

RESULTS — The time required to form aggregates was shorter in diabetic patients at shear rates below 200 s^{-1} ($P < 0.01$). Erythrocytes formed larger aggregates in diabetic patients than in control subjects ($P < 0.05$ at 2 to 100 s^{-1}).

CONCLUSIONS — Ultrasound can potentially noninvasively demonstrate, in vivo and in situ, the impact of local abnormal EA on arteriovenous flow disorders in diabetes.

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Flow disorders in diabetes often lead to severe outcomes in various organs and tissues; abnormal rheology of erythrocytes (RBC) likely impairs macro- and microcirculatory blood flow, tissue oxygenation, and vascular tone regulation in affected patients (1–3). Diabetic retinopathy is attributed to microvascular flow disorders and enhanced RBC aggregation (4). Erythrocyte aggregation (EA) and plasma viscosity are also predictive of diabetic foot syndrome deterioration (5). EA is a reversible phenomenon responsible for increased blood viscosity at low shear rates. RBC hyperaggregation can also promote flow stasis and thrombosis in macrocirculation. This study proposes an ultrasound method that has the potential to noninvasively detect early rheological disorders in situ in blood vessels. The method is based on backscattering of ultrasound by blood; it measures the extent of EA and its shear rate dependency.

RESEARCH DESIGN AND METHODS

Populations

Recruited individuals were nonsmoking males. They completed a questionnaire on current medications and medical history. BMI and blood pressure were measured. Nine patients with type 2 diabetes and eight healthy control subjects gave informed consent to the approved protocol. Patients with poor metabolic control were intentionally chosen. Lipid profile and inflammatory proteins (fibrinogen, Von Clauss method; haptoglobin and immunoglobin G, immunonephelometric method; and C-reactive protein, latex agglutination technique) were determined for each participant.

Six patients were on oral antidiabetic and aspirin medications, three were on insulin, five were on cholesterol-lowering therapy, and four were treated for hypertension. Those on insulin had a history of coronary artery disease, and two of them had suffered from myocardial infarction. Distal angiopathy or cutaneous trophic disorders were not present. Mean ± SD age of patients was 58.3 ± 8.8 years (range 41–70). Healthy subjects were age matched (51.1 ± 8.7 years [range 41–64]), did not take regular medications, and had no history of cardiovascular disease. None had lipid disorders or hypertension.

Couette experiments

A Couette instrument made of two concentric cylinders and installed in an incubator at 37°C generated homogeneous shear rates via a rotating outer cylinder. Fifty milliliters of EDTA anticoagulated blood at 40% hematocrit was introduced between both cylinders. The stationary inner cylinder held an ultrasound transducer perpendicular to flow. The 32-MHz polyvinylidine flouride ultrasound transducer (Visualsonics, Toronto, Canada) had a −6 dB bandwidth of 15–45 MHz. It was pulsed with bipolar square waves (model no. AVB2-TA-C-CRIMA; Avtech, Ottawa, Canada). Received radio frequency (RF) echoes (model no. AU-3A-0120; Miteq, Hauppauge, NY) were amplified by 54 dB, filtered between 10 and 50 MHz (model no. 5900 PR; Panametrics, Waltham, MA), and digitized at 250 MHz (model no. 8500 CS; GageScope, Montreal, Canada).

The protocol consisted of imposing a 500 s^{-1} shear rate for 120 s to disrupt aggregates. Then, aggregation kinetics were recorded for 380 s at randomly applied reduced shear rates of 0, 1, 2, 10, 50, 100, and 200 s^{-1}. One hundred RF echoes were acquired every 2 s. For each blood sample, the protocol was repeated three times for averaging. RF signals were adjusted to compensate for blood attenuation at each shear rate and integrated backscatter coefficient (IBSC) was deter-

From the [1]Laboratory of Biorheology and Medical Ultrasonics, Research Center, University of Montreal Hospital, Montreal, Quebec, Canada; the [2]Department of Radiology, Radio-Oncology and Nuclear Medicine and the Institute of Biomedical Engineering, University of Montreal, Quebec, Canada; and the [3]Research Center, University of Montreal Hospital, Montreal, Quebec, Canada, and the Department of Medicine, University of Montreal, Montreal, Quebec, Canada.
Corresponding author: Dr. Guy Cloutier, guy.cloutier@umontreal.ca.
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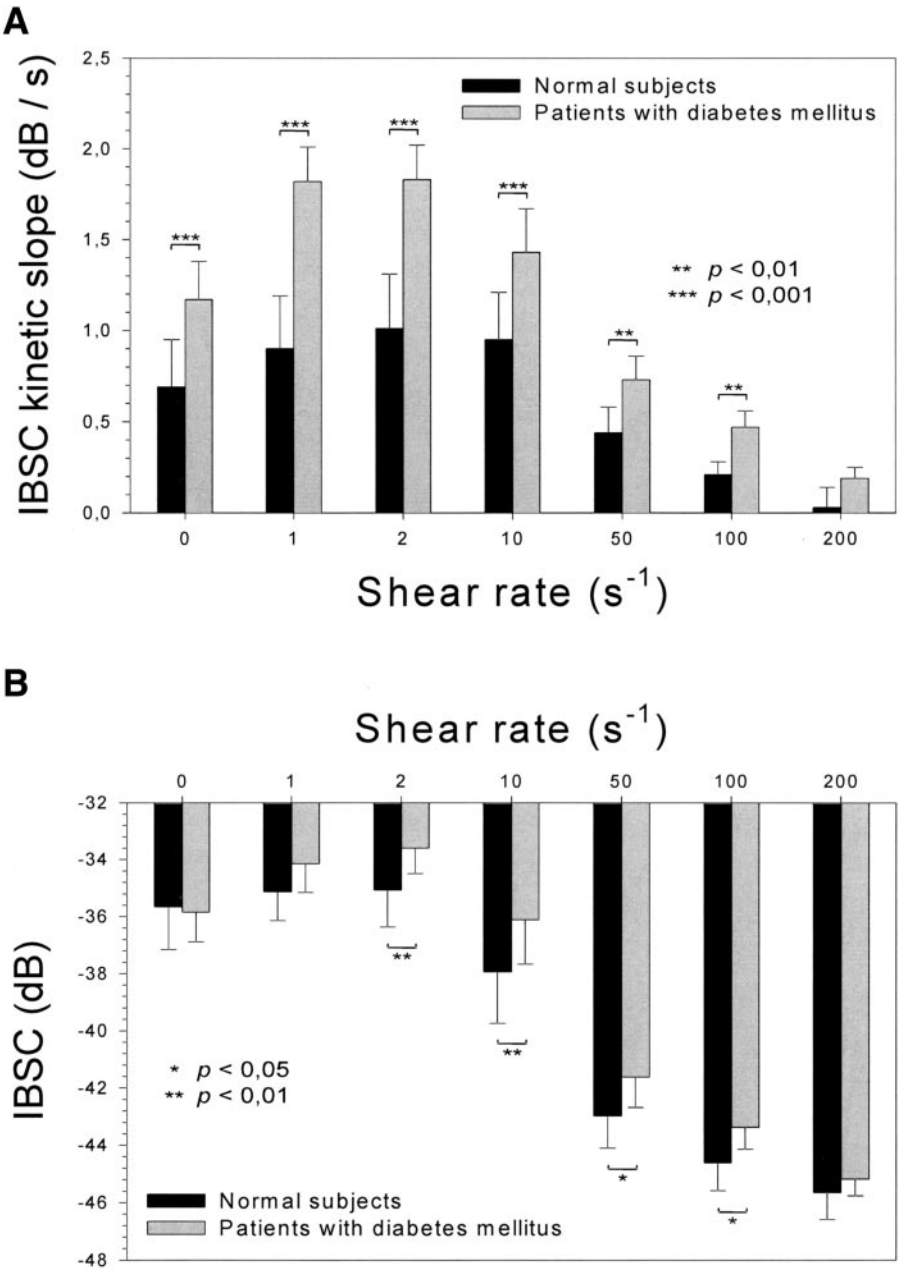

Figure 1—A: *Raising slopes from 2 to 8 s of the IBSC as a function of the shear rate applied to blood samples (means ± SD).* B: *IBSC at the plateau of RBC aggregation as a function of the shear rate. The power of 0 dB corresponds to that of a perfect flat stainless steel reflector. Two-way analyses of variance (Tukey method for multiple comparisons) confirmed impact of shear rate* (P < *0.001) and population* (P < *0.001) on IBSC slopes and IBSC at plateaus. The* P *values shown on the figure correspond to multiple comparisons between populations. IBSC slopes at 2 s^{-1} were correlated with physiological variables (Pearson coefficient* r = *0.59,* P = *0.02 for A1C;* r = *0.53,* P = *0.03 for fibrinogen;* r = *0.54,* P = *0.02 for immunoglobin G; and* r = *0.72,* P = *0.001 for haptoglobin). Forward-stepwise regressions explained IBSC kinetic slopes at 2 s^{-1} by the following model* (r = *0.94): IBSC kinetic* = −*1.00* (P = *0.009*) + *0.67 haptoglobin* (P < *0.001*) + *0.11 immunoglobin G* (P = *0.003*) + *5.60 A1C* (P = *0.048*). *Only immunoglobin G was positively correlated with the plateau of IBSC at 2 s^{-1} (Pearson coefficient* r = *0.49,* P = *0.046).*

mined as previously described (6). IBSC reflects the number of RBC per aggregate (7).

RESULTS — BMI, blood pressure, triglycerides, total cholesterol, HDL and LDL cholesterol, fibrinogen, and C-reactive protein were not different between groups ($P > 0.05$; unpaired t tests), whereas immunoglobin G (8.4 ± 1.0 vs. 11.3 ± 1.8 g/l, $P < 0.001$), haptoglobin (1.0 ± 0.4 vs. 1.6 ± 0.5 g/l, $P < 0.05$), and A1C (5.5 ± 0.8 vs. 8.8 ± 2.1%, $P < 0.01$) were significantly higher in diabetic patients than in control subjects. Couette flow protocol resulted in disaggregation of RBC and minimum IBSC at 500 s^{-1}, formation of aggregates and rapid increase in IBSC depending on reduced applied shear (Fig. 2 of ref. 7), and development of stable aggregate sizes and plateaus of IBSC after a few seconds for most shears to minutes at 0 s^{-1}.

Figure 1*A* summarizes mean raising slopes of IBSC between 2 and 8 s after shear rate reductions. In control subjects, maximum slope at 2 s^{-1} was not different from that at 1 and 10 s^{-1} ($P > 0.89$), whereas in diabetic patients, maximum slope of IBSC occurring at 2 s^{-1} was similar to that at 1 s^{-1} ($P = 1.0$). Except for that at 200 s^{-1} ($P = 0.11$), kinetic slopes were always faster in diabetic patients, which is indicative of higher rates of neighboring RBC clustering.

IBSC at plateaus averaged between 180 and 380 s after shear rate reductions as presented in Fig. 1*B*. In control subjects, maximum IBSC at 2 s^{-1} was similar to those at 0 and 1 s^{-1} ($P > 0.95$). Similarly, IBSCs at 1 and 2 s^{-1} were similar in diabetic patients ($P > 0.95$). IBSCs were statistically higher in diabetic patients between 2 and 100 s^{-1}, which reflects stronger adhesions and bigger steady-state aggregate sizes in diabetes.

CONCLUSIONS — Statistically significant differences in Fig. 1 were noted for shears between 2 and 100 s^{-1}, which correspond to normal flow at center streams and pathological flow stasis in recirculation zones of large systemic veins and arteries. Accordingly, EA in diabetes can be related to lower-limb artery ischemic events, microangiopathy in foot extremities, and retinopathy. Inflammation is involved in pathogenesis of type 2 diabetes and RBC aggregation, which agrees with our results (legend of Fig. 1). Subacute inflammatory state promoting RBC aggregation is also associated with obesity (8) and metabolic syndrome (9). Thus, reducing inflammation (and indirectly aggregation) with statins and A1C with antidiabetic medication and/or diet are indicated because both have known benefits to cardiovascular consequences of diabetes. We reported measurements from a laboratory instrument, but a short-term objective is sizing RBC aggregates in vivo with ultrasound (10). At 32 MHz, superficial (5–6 mm depth) vessels can be scanned. The proposed noninvasive method should be

investigated further because it may have potential benefit for diagnosis and follow-up of diabetic foot complications and for monitoring therapy.

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