Original Article

Loss of Endothelial Glycocalyx During Acute Hyperglycemia Coincides With Endothelial Dysfunction and Coagulation Activation In Vivo

Max Nieuwdorp,[1] Timon W. van Haeften,[2] Mirella C.L.G. Gouverneur,[3] Hans L. Mooij,[1] Miriam H.P. van Lieshout,[1] Marcel Levi,[4] Joost C.M. Meijers,[1] Frits Holleman,[4] Joost B.L. Hoekstra,[4] Hans Vink,[3] John J.P. Kastelein,[1] and Erik S.G. Stroes[1]

Hyperglycemia is associated with increased susceptibility to atherothrombotic stimuli. The glycocalyx, a layer of proteoglycans covering the endothelium, is involved in the protective capacity of the vessel wall. We therefore evaluated whether hyperglycemia affects the glycocalyx, thereby increasing vascular vulnerability. The systemic glycocalyx volume was estimated by comparing the distribution volume of a glycocalyx permeable tracer (dextran 40) with that of a glycocalyx impermeable tracer (labeled erythrocytes) in 10 healthy male subjects. Measurements were performed in random order on five occasions: two control measurements, two measurements during normoinsulinemic hyperglycemia with or without *N*-acetylcysteine (NAC) infusion, and one during mannitol infusion. Glycocalyx measurements were reproducible (1.7 ± 0.2 vs. 1.7 ± 0.3 l). Hyperglycemia reduced glycocalyx volume (to 0.8 ± 0.2 l; $P < 0.05$), and NAC was able to prevent the reduction (1.4 ± 0.2 l). Mannitol infusion had no effect on glycocalyx volume (1.6 ± 0.1 l). Hyperglycemia resulted in endothelial dysfunction, increased plasma hyaluronan levels (from 70 ± 6 to 112 ± 16 ng/ml; $P < 0.05$) and coagulation activation (prothrombin activation fragment 1 + 2: from 0.4 ± 0.1 to 1.1 ± 0.2 nmol/l; D-dimer: from 0.27 ± 0.1 to 0.55 ± 0.2 g/l; $P < 0.05$). Taken together, these data indicate a potential role for glycocalyx perturbation in mediating vascular dysfunction during hyperglycemia. ***Diabetes*** **55:480–486, 2006**

Patients with diabetes have increased vascular vulnerability to atherogenic insults, leading to accelerated atherogenesis. Although atherogenesis is in part due to the increased prevalence of traditional cardiovascular risk factors, these factors cannot fully explain the propensity toward vascular complications in diabetic patients (1). Hyperglycemia itself has been shown to induce a wide array of downstream effects that adversely affect the protective capacity of the vessel wall (2). Hyperglycemia has been associated with enhanced endothelial permeability, increased leukocyte-endothelium adhesion, and impaired nitric oxide (NO) bioavailability (3–5). Despite clear progress in understanding the underlying pathophysiological mechanisms contributing to this vascular dysfunction, it has proven difficult to unravel a final common pathway for the increased vascular vulnerability under hyperglycemic conditions (6).

The glycocalyx covers the endothelium and consists of endothelial cell–derived proteoglycans, glycoproteins, and adsorbed plasma proteins. This layer has been shown to orchestrate vascular homeostasis (7). Its thickness (up to 1 μm) may explain its potent antiadhesive effects on leukocytes and platelets (8,9). Hyaluronan glycosaminoglycans, one of the major constituents of the glycocalyx, are crucial for maintaining endothelial barrier properties for plasma macromolecules (10). The glycocalyx also serves as a mechanosensor of shear stress, mediating shear-induced release of NO by endothelial cells (11–13). In fact, selective perturbation of the glycocalyx leads to increased vascular permeability, attenuated NO availability, and increased adhesion of leukocytes and platelets. Reconstitution of the glycocalyx results in the restoration of its barrier and antiadhesive properties (10,14). In view of the intricate relation between glycocalyx integrity and vascular homeostasis in experimental models, it has been postulated that glycocalyx derangement could contribute to increased vascular vulnerability in humans (15). Because increased degradation of proteoglycans has previously been demonstrated in hyperglycemic conditions (16,17), the impact of hyperglycemia on the glycocalyx merits special interest. In the present study, we set out to evaluate the impact of hyperglycemia on the glycocalyx in healthy volunteers. We measured changes in the systemic glycocalyx volume before and 6 h after hyperglycemic-normoinsulinemic clamping. We simultaneously assessed changes in plasma hyaluronan, endothelial function, and coagulation parameters. To elucidate the role of reactive oxygen species, we repeated the hyperglycemic clamp in conjunction with the infusion of the antioxidant *N*-acetylcysteine (NAC).

RESEARCH DESIGN AND METHODS

Subjects for this study were 10 Caucasian male volunteers. Measurements were performed on five separate occasions in random order, with a minimum

From the [1]Department of Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands; the [2]Department of Internal Medicine, University Medical Center Utrecht, Utrecht, the Netherlands; the [3]Department of Medical Physics, Academic Medical Center, Amsterdam, the Netherlands; and the [4]Department of Internal Medicine, Academic Medical Center, Amsterdam, the Netherlands.

Address correspondence and reprint requests to Erik S.G. Stroes, MD, PhD, Department of Vascular Medicine, Academic Medical Center, Meibergdreef 9, Rm. F4-159.2, 1105 AZ, Amsterdam, Netherlands. E-mail: e.s.stroes@amc.uva.nl.

Received for publication 24 August 2005 and accepted in revised form 25 October 2005.

FMD, flow-mediated dilation; NAC, *N*-acetylcysteine.

interval of 2 weeks between measurements. Two baseline measurements were performed after saline infusion to estimate the intersession coefficient of variance (CV) of the glycocalyx volume estimates. Two measurements were performed after hyperglycemic clamping and mannitol infusion, respectively. To gain insight into the mechanistic properties, a fifth measurement was performed after a hyperglycemic-normoinsulinemic protocol with concomitant infusion of NAC in a random subgroup ($n = 6$).

The protocol was approved by the internal review board and written informed consent was obtained from all volunteers before the investigation. The study was carried out in accordance with the principles of the Declaration of Helsinki.

Estimation of glycocalyx volume. The endothelial glycocalyx limits access to plasma macromolecules and erythrocytes (18,19). Hence the glycocalyx volume can be estimated by comparing the circulating blood volume using a glycocalyx impermeable tracer such as labeled erythrocytes (18,20) with the total intravascular volume using a glycocalyx permeable tracer such as neutral dextran 40 (40 kDa). For the study, centrifuged erythrocytes were mixed with sodium fluorescein (250 mg/ml) for 5 min. After being washed, the labeled erythrocytes were resuspended in saline to the initial volume and reinfused. Blood was subsequently drawn at 4, 5, 6, and 7 min after infusion. The fraction of labeled erythrocytes versus the total erythrocyte pool was used to estimate circulating erythrocyte volume. Unlabeled erythrocytes obtained before the injection ($t = -1$ min) served as negative controls. Labeled erythrocytes were measured using a FACScan analyzer (FACSCalibur; Becton Dickinson, Mountain View, CA), with at least 100,000 cells being counted to measure the circulating fraction of labeled erythrocytes. Data were analyzed using Cellquest (Becton Dickinson, San Jose, CA). The circulating plasma volume was calculated as $[(1 - Hsys) \times Vrbc]/Hsys$, where *Vrbc* is the circulating erythrocyte volume and *Hsys* is the large vessel hematocrit (20).

Dextran 40 is used as a probe for intravascular glycocalyx volume (19). Before dextran 40 was injected, a single bolus of 10 ml dextran 1 (Promiten; NPBI, Emmercompascuum, the Netherlands) was injected to attenuate the risk for anaphylactic reactions. At least 1 h later, 100 ml dextran 40 (Rheomacrodex; NPBI) was injected intravenously, after which repeated blood sampling at 5, 7, 10, 15, 20, and 30 min was performed. The dextran 40 concentration was calculated by measuring the increase in glucose concentration in the postinfusion samples after hydrolyzation of dextran glucose polymers (21). The glucose concentration per time point was assessed in duplicate using the hexokinase method (Gluco-quant and Hitachi 917; Hitachi). The procedure was calibrated with dextran 40 added to plasma in vitro. To determine the initial intravascular distribution volume of dextran 40, the concentration of dextran 40 at the time of injection was estimated by exponential fitting of the measured dextran 40 concentrations.

Hyperglycemic-normoinsulinemic clamp. A hyperglycemic clamp was applied for 6 h with a target glucose concentration of 16 mmol/l (300 mg/dl) (22). To prevent hypokalemia, 10 mmol/l KCl was added to the glucose solution. Octreotide (Sandostatin; Novartis, Basel, Switzerland) was dissolved in saline and albumin and administered at a final concentration of 30 $ng \cdot kg^{-1} \cdot min^{-1}$ to attenuate the increase in endogenous insulin production and minimize potential confounding effects of hyperinsulinemia (23). At this dosage, octreotide has no significant vasoactive or hemostatic side effects (22,24). During the clamping protocol, the blood glucose concentration was measured by the glucose oxidase method (YSI 2300 STAT; YSI, Yellow Springs, OH). The target value of glucose was maintained by adjusting the infusion rate of 20% glucose (Baxter). As a time and osmolality control, the octreotide protocol was repeated on a separate study day, during which glucose 20% was replaced with an equimolar 20% mannitol (Baxter) infusion. Venous samples were obtained throughout the protocol to document the achieved level of osmolality. Osmolality was determined by measuring the freezing point depression on the Osmo Station (Menarini Benelux). All samples for glucose, insulin, and osmolality were performed in duplicate. The glutathione donor NAC (clinically graded and manufactured by the Department of Pharmacy at the Academic Medical Center, Amsterdam, the Netherlands) was administered as a bolus of 100 mg/kg in 15 min before the start of the glucose infusion and thereafter as a continuous infusion of 60 mg/kg throughout the identical hyperglycemia study protocol. The rate of infusion and total amount of infused NAC in our experiment was similar to that used for the treatment of acetominophen intoxication (25).

Flow-mediated dilation. Flow-mediated dilation (FMD) was assessed before each glycocalyx volume measurement (26). With the subjects in the supine position, a blood pressure cuff was placed just below the elbow of the right arm. The brachial artery in the right antecubital fossa was visualized using a 7.5-MHz transducer. A wall tracking system was used to measure lumen diameter. After two baseline vessel diameter measurements were obtained, reactive hyperemia was induced by inflating the lower-arm blood pressure cuff to 200 mmHg. Upon release of the cuff after 4 min, ultrasonography continued for 5 min to allow for lumen diameter measurements at 30-s intervals. Images were stored digitally and analyzed off-line using the Wall Track System software analysis package. All measurements were performed by the same person, who was unaware of clinical details and the stage of the experiment. At our institution, intra- and intersession CVs for baseline diameter assessment using the Wall Track System are 1.1 and 3.8%, respectively. Intersession variability of the FMD measurement is 13.9% (26).

TABLE 1
Clinical characteristics of the volunteers

n	10
Sex (male/female)	10/0
Age (years)	25.3 ± 2.6
BMI (kg/m^2)	22.5 ± 1.4
Systolic blood pressure (mmHg)	122 ± 11
Diastolic blood pressure (mmHg)	65 ± 4
Smoking (yes/no)	0/10
Heart rate (bpm)	56 ± 7
Total cholesterol (mmol/l)	3.6 ± 0.5
LDL cholesterol (mmol/l)	2.0 ± 0.4
HDL cholesterol (mmol/l)	1.2 ± 0.2
Triglycerides (mmol/l)	0.6 ± 0.3
Fasting glucose (mmol/l)	4.8 ± 0.3
Fasting insulin (pmol/l)	35 ± 14
Aspartate aminotransferase (unit/l)	23 ± 8
Alanine aminotransferase (unit/l)	22 ± 7
HbA_{1c} (%)	5.3 ± 0.2
High-sensitivity C-reactive protein (mg/l)	0.5 ± 0.2

Data are means ± SD.

Blood sampling and laboratory methods. Blood samples were drawn from the subjects after a 12-h overnight fast and 2, 4, 6, and 30 h after the start of the infusion. Aliquots were centrifuged within 1 h after being collected, snap-frozen in liquid nitrogen, and stored at −80°C. Hematocrit was measured after centrifugation of heparinized blood in a Hettich-Hematokrit centrifuge (Hettich, Tuttlingen, Germany) at 10,000 rpm for 5 min. Total cholesterol, HDL cholesterol, and triglycerides were measured by standard enzymatic methods (Roche Diagnostics, Basel, Switzerland). LDL cholesterol was calculated using the Friedewald formula. Alanine and aspartate aminotransferase were measured by a pyridoxalphosphate activation assay (Roche Diagnostics). HbA_{1c} was measured by high-performance liquid chromatography (Reagens; Bio-Rad, Veenendaal, the Netherlands) on Variant II (Bio-Rad). Quantitative plasma hyaluronan levels were measured in duplicate by enzyme-linked immunosorbent assay (Echelon Biosciences, Salt Lake City, UT). As a measure of thrombin generation, the prothrombin activation fragment F1 + 2 (Dade Behring, Marburg, Germany) was assessed by enzyme-linked immunosorbent assay. D-Dimer levels were used as a reflection of endogenous fibrinolysis in the presence of coagulation activation and were measured with an automated quantitative latex particle immunoassay (Biomerieux, Durham, NC). Plasma insulin was measured by an immunoluminimetric assay (Immulite insuline) on Immulite 2000 (Diagnostic Product).

Statistical analysis. All data except Table 1 are presented as means ± SE. Differences between treatment groups were tested by ANOVA for repeated measures. Comparisons within groups were done with Wilcoxon's signed-rank test. $P < 0.05$ was considered significant.

RESULTS

Reproducibility of glycocalyx measurement. Baseline characteristics of volunteers are listed in Table 1. Throughout all infusion protocols, blood pressure and heart rate remained unaffected (data not shown). Infusion of the dextran 40 solution had no significant effect on hematocrit values. Circulating plasma (3.0 ± 0.1 vs. 3.0 ± 0.1 l; NS) and systemic dextran 40 distribution (4.7 ± 0.2 vs. 4.7 ± 0.3 l; NS) volumes were similar during the two baseline study visits. Accordingly, glycocalyx volumes were reproducible between visits (1.7 ± 0.2 vs. 1.7 ± 0.3 l; NS; intersession CV 15.2 ± 9.8%) (Fig. 1*A* and *B*).

Glycocalyx volume after 6-h infusion of glucose, NAC with glucose, or mannitol. During hyperglycemic clamping, the glucose concentration was raised to target levels within 15 min and remained stable throughout the hyper-

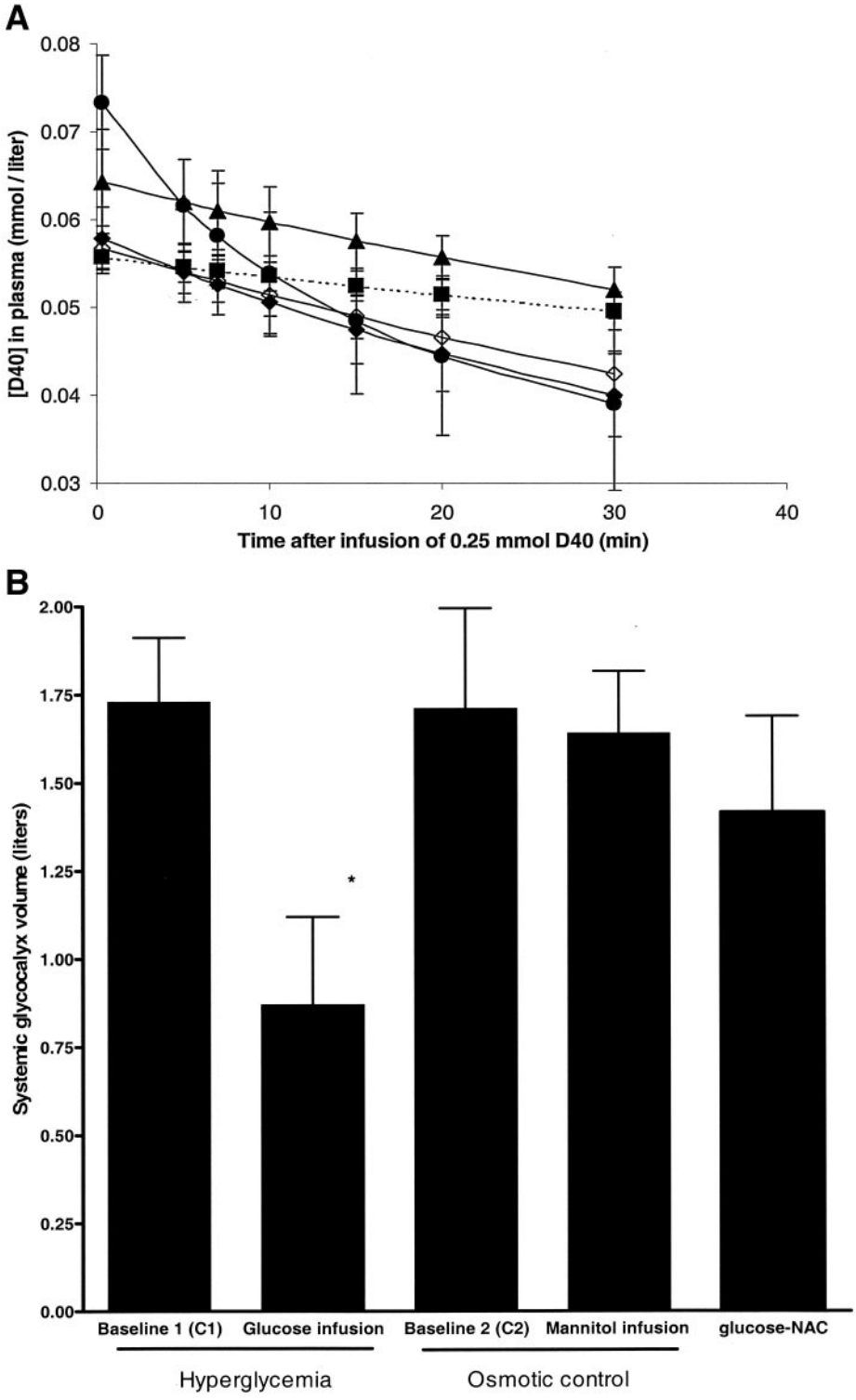

FIG. 1. ***A*: Plasma dextran 40 (D40) clearance curves under baseline (◇, $[Dex40]_{C1} = 0.057e^{-0.000481\,t}$; ◆, $[Dex40]_{C2} = 0.058e^{-0.000671\,t}$), glucose infusion (●, $[Dex40]_{HG} = 0.073e^{-0.001718\,t}$), glucose-NAC infusion (▲, ($[Dex40]_{NAc} = 0.064e^{-0.000320\,t}$), and mannitol infusion (■, $[Dex40]_{Man} = 0.056e^{-0.000190\,t}$). During glucose infusion, the rate of dextran 40 plasma clearance was increased as compared with the mannitol infusion. Depicted values on each time point are expressed as means ± SE. *B*: Systemic glycocalyx volumes were determined in random order before and after infusion with glucose (*bars 1* and *2*) or mannitol (*bars 3* and *4*) and after glucose-NAC (*bar 5*). Systemic glycocalyx volumes were identical at baseline; glucose infusion resulted in a statistically significant decrease in systemic glycocalyx volume compared with baseline, mannitol, and glucose-NAC. Data are means ± SE. *$P < 0.05$.**

glycemic period (~16 mmol/l). Glucose levels during mannitol infusion were unchanged (4.8 ± 0.1 vs. 5.3 ± 0.1 mmol/l; NS). Despite coinfusion of octreotide, plasma insulin levels rose during hyperglycemia but remained within the physiological range (from 35 ± 5 to 116 ± 10 pmol/l; $P < 0.001$). Mannitol infusion with octreotide was associated with a decrease in insulin levels (from 42 ± 5 to 15 ± 1 pmol/l; $P < 0.01$). The plasma osmolality levels during hyperglycemia and mannitol were comparable (284 ± 2 vs. 288 ± 2 mmol/kg; NS). Glycocalyx volumes were profoundly decreased during hyperglycemia versus with mannitol (0.8 ± 0.2 vs. 1.6 ± 0.1 l; $P < 0.05$) (Fig. 1*B*), predominantly due to a reduction in the dextran 40 distribution volume (4.0 ± 0.3 vs. 4.7 ± 0.3 l; hyperglycemia vs. mannitol, $P < 0.05$). There were no changes in circulating plasma volumes (3.2 ± 0.1 vs. 3.1 ± 0.1 l; hyperglycemia vs. mannitol, NS) or in hematocrit values (0.40 ± 0.001 vs. 0.41 ± 0.001%; hyperglycemia vs. mannitol, NS). Coinfusion of NAC during the hyperglycemic clamp abolished the reduction in glycocalyx volume (1.4 ± 0.2 l; $P < 0.05$ vs. hyperglycemia alone) due to normalization of the dextran 40 distribution volume (4.4 ± 0.2 l) without affecting

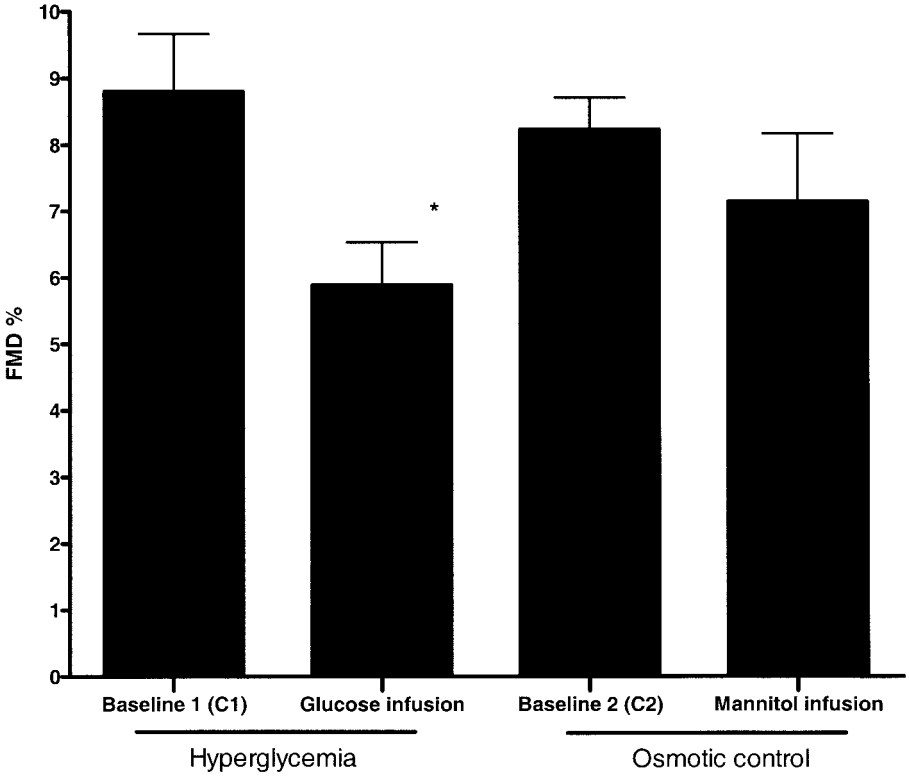

FIG. 2. FMD determined in a random order before and after infusion with glucose (*bars 1* and *2*) or mannitol (*bars 3* and *4*). FMD was identical before the interventions; glucose infusion resulted in a statistically significant decrease in FMD compared with mannitol infusion and baseline. Data are means ± SE. *$P < 0.05$.

circulating plasma volume (3.0 ± 0.1 l) and hematocrit (0.41 ± 0.01%).

Endothelial function. FMD showed good reproducibility between saline visits (8.8 ± 0.8 vs. 8.2 ± 0.5%; NS; intersession CV 16.8 ± 8.2%). FMD was attenuated during hyperglycemia (5.8 ± 0.6 vs. 8.8 ± 0.8%; hyperglycemia vs. baseline, $P < 0.05$). Mannitol infusion had no effect on FMD (7.1 ± 1.0 vs. 8.2 ± 0.5%; mannitol vs. baseline, NS) (Fig. 2). There was no difference in nitroglycerine response after hyperglycemia compared with after mannitol infusion (data not shown). Of note, due to the small sample size, we did not determine FMD in the NAC hyperglycemia protocol.

Laboratory parameters. Plasma hyaluronan levels rose significantly during hyperglycemia (112 ± 16 vs. 70 ± 6 ng/ml; hyperglycemia vs. baseline, $P < 0.05$) and returned toward baseline values within 24 h (81 ± 6 ng/ml) (Fig. 3). Activation of the coagulation (as indicated by an increase in F1 + 2 levels from 0.4 ± 0.1 to 1.1 ± 0.2 nmol/l; $P < 0.05$) and fibrinolytic (increase in D-dimer levels from 0.27 ± 0.1 to 0.55 ± 0.2 g/l; $P < 0.05$) systems occurred during hyperglycemia (Fig. 4*A* and *B*). No effect of mannitol was seen on these parameters. Hyperglycemia with concomitant NAC infusion resulted in blunting of plasma hyaluronan shedding (69 ± 8 ng/ml; $P < 0.05$) (Fig. 3) and coagulation activation (F1 + 2: 0.8 ± 0.15 nmol/l; D-dimer: 0.44 ± 0.07 g/l; NS) compared with hyperglycemia (Fig. 4*A* and *B*).

DISCUSSION

In the present study, we showed that the glycocalyx constitutes a large intravascular compartment in healthy volunteers that can be estimated in a reproducible fashion in vivo. More importantly, we showed that hyperglycemic clamping elicits a profound reduction in glycocalyx volume that coincides with increased circulating plasma levels of glycocalyx constituents like hyaluronan, an observation that is consistent with the release of glycocalyx constituents into the circulation. These disturbances are accompanied by impaired FMD as well as activation of the coagulation system. Infusion of the antioxidant NAC prevented this glycocalyx perturbation, indicating that the generation of reactive oxygen species contributes to the glycocalyx perturbation under hyperglycemic conditions.

Hyperglycemia and glycocalyx volume. Previously we validated glycocalyx measurements in isolated vessels by comparing erythrocyte and dextran 40 distribution volumes as markers of glycocalyx impermeable and permeable tracers, respectively (18,19). Consistent with these experimental data, we now find comparable values for glycocalyx volume in healthy volunteers with good reproducibility of between-session measurements (CV <20%). The size of the glycocalyx volume in the present study was in line with predictions of glycocalyx dimension in vivo, based on a thickness of 0.5–3.0 μm combined with a total endothelial surface area of 1,000–7,000 m^2 (27,28). After 6 h of hyperglycemic clamping, the systemic glycocalyx volume was reduced to ~50% of the baseline value. This reduction coincided with a rapid increase in circulating plasma levels of hyaluronan, an important constituent of the glycocalyx. Similarly, hyperglycemia has been associated with increased hyaluronidase activity and concomitant increased plasma hyaluronan concentrations in animal models (17,29). Hyaluronan has been shown to be a principal determinant of vascular permeability because selective removal of hyaluronan from the vessel wall is accompanied by a profound increase in macromolecular glycocalyx permeation (10). We showed in vivo that the loss of glycocalyx volume and the shedding of hyaluronan into plasma is indeed accompanied by a significant increase in the rate of dextran 40 clearance from circulation (Fig. 1*A*). This correlation between glycocalyx volume

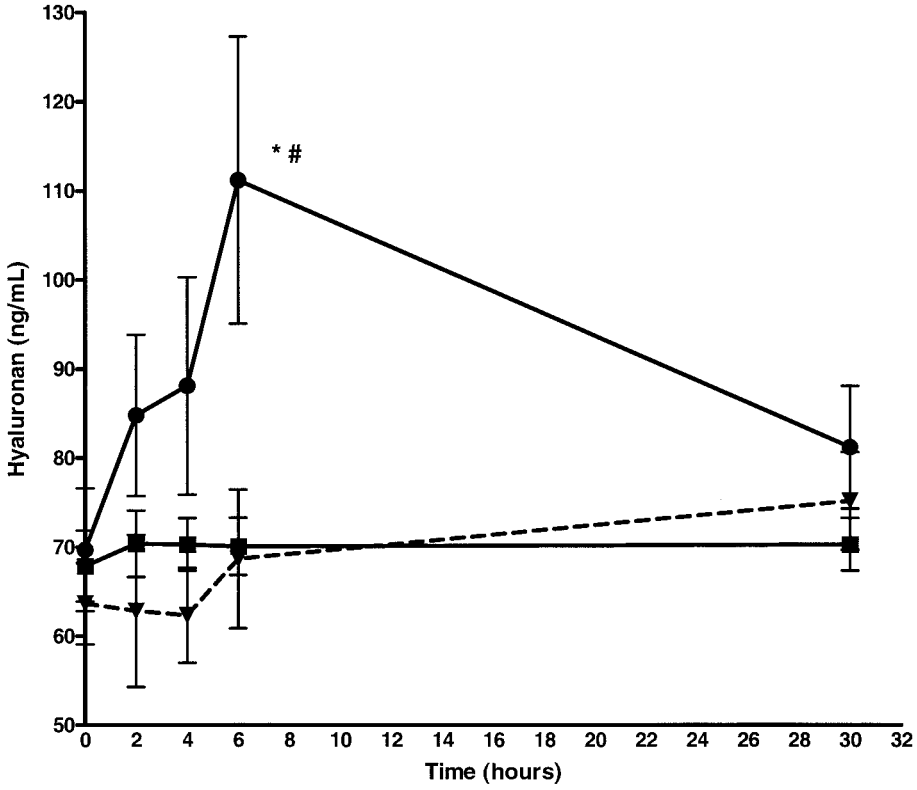

FIG. 3. Shedding of endothelial glycocalyx compounds (as assessed by plasma hyaluronan) in subjects infused with glucose (●), mannitol (■), or glucose-NAC (▲). Data are means ± SE. *$P < 0.05$ vs. baseline; #$P < 0.05$ among groups.

reduction and increased permeability suggests a potential contribution of the glycocalyx, particularly hyaluronan, to the preservation of the systemic vascular permeability barrier.

Increased formation of reactive oxygen species and glycocalyx. Several mechanisms may contribute to the loss of glycocalyx volume during acute hyperglycemia. First, hyperglycemia per se is a potent pro-oxidant and proinflammatory stimulus that has been linked to enhanced degradation of the glycocalyx as well as to shedding of hyaluronan (30). Therefore, glycocalyx loss may be secondary to a direct effect of oxygen radicals on the synthesis of glycosaminoglycans. Indeed, in our study, infusion of the potent antioxidant NAC abolished the reduction in glycocalyx during hyperglycemia. On the other hand, increased shedding of glycosaminoglycans may follow vascular injury, resulting in an upregulation of glycosaminoglycan synthesis to compensate for stimulated increased degradation (6,31).

Endothelial function. In conjunction with glycocalyx loss we observed a loss of FMD after hyperglycemic clamping. In line with this observation, several research groups have reported endothelial dysfunction under hyperglycemic conditions (22,32). Although impaired NO bioavailability has been attributed predominantly to direct inactivation of NO by increased radical production (33,34), the present finding provides us with an alternative explanation. It has been demonstrated that the endothelial glycocalyx plays an important mechanosensory role by translating intravascular shear stress into biochemical activation of endothelial cells (12). Accordingly, the release of NO in response to shear stress is abolished after enzymatic removal of glycosaminoglycans from the endothelial glycocalyx (15).

Coagulation activation. Hyperglycemia elicited coagulation and fibrinolysis, as reflected by the increased thrombin generation (F1 + 2) as well as increased fibrinolysis (D-dimer). Our data are in line with those from studies showing that induction of acute hyperglycemia in healthy volunteers increases plasma levels of coagulation factor VIIa and stimulates tissue factor–dependent activation of coagulation (35). The endothelial glycocalyx is a crucial compartment for binding and regulating enzymes involved in the coagulation cascade. In addition, the most important inhibitor of thrombin and factor Xa (i.e., antithrombin) is firmly attached to the endothelium (36). In support of this, we and others have previously demonstrated that glycocalyx perturbation has direct effects on coagulation and fibrinolytic responses (9,37). It is therefore not surprising that hyperglycemia-induced loss of glycocalyx is accompanied by activation of coagulation. The subsequent increase in endogenous fibrinolysis can thus be explained by counterbalancing the increased thrombin generation during hyperglycemia.

Study limitations. The accuracy of glycocalyx volume estimates in our study was determined by the accuracy of dextran 40 distribution volume estimates. Because of its relatively small size and neutral charge, dextran 40 is cleared from the circulation slowly. Extrapolation of measured plasma concentrations of dextran 40 to the time of its initial intravascular injection is used to estimate intravascular dextran 40 concentration before leakage. As can be appreciated from the average clearance curves in Fig. 1*A*, the error of the estimated initial dextran 40 concentration was relatively small and therefore had no major impact on the estimates of glycocalyx volume. Second, the stable circulating blood volumes during hyperglycemic clamping cannot exclude changes of microcirculatory volume. Because of anatomical dimensions, the largest part of the erythrocyte volume is located in the macrovasculature, whereas the measured dextran 40 volume was mainly situated in the microvasculature. In fact, we re-

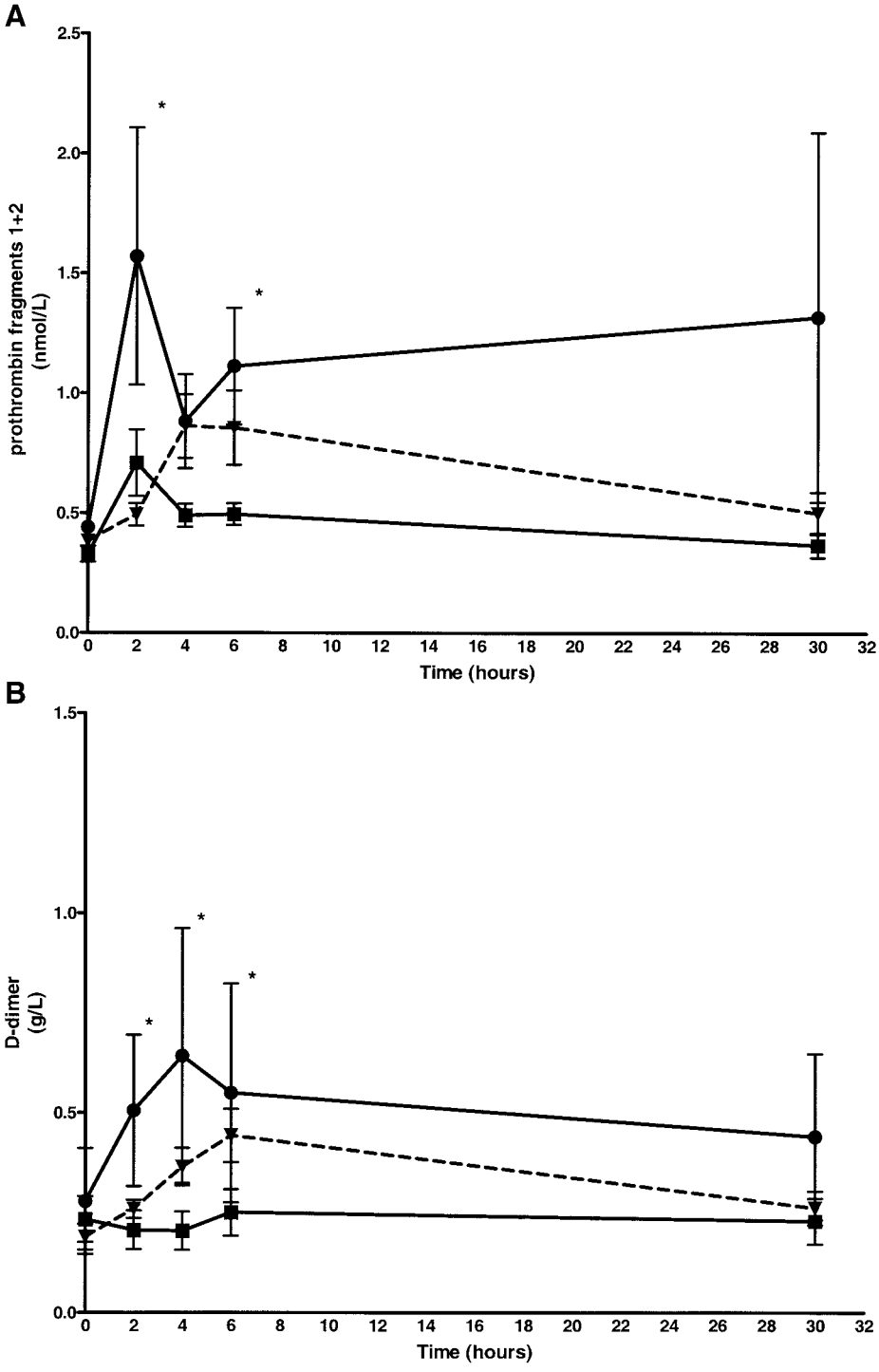

FIG. 4. ***A*****: Activation of coagulation system (as assessed by prothrombin fragments 1 + 2) in human volunteers infused with glucose (●), mannitol (■), or glucose-NAC (▲). Data are means ± SE. ****P*** **< 0.05 vs. baseline.** ***B*****: Activation of the fibrinolytic system (as assessed by D-dimer levels) in subjects infused with glucose (●), mannitol (■), or glucose-NAC (▲). Data are means ± SE. ****P*** **< 0.05 vs. baseline.**

cently reported that hyperglycemia reduces perfused murine capillary density by up to 38% (38). Hence, in addition to glycocalyx shedding, impaired microcirculatory perfusion may also have contributed to the reduction of systemic glycocalyx. Finally, during hyperglycemic clamping, an increase in insulin levels was observed despite the concomitant infusion of octreotide. Higher octreotide administration was not feasible due to gastrointestinal side effects (23). Under these circumstances, plasma insulin increases are inextricably entangled with hyperglycemic clamping in humans (22). Although the insulin levels were within the physiological range, we cannot exclude a potential confounding effect of insulin.

Clinical implications. Experimental studies have shown that the glycocalyx is a crucial intravascular compartment that mediates transduction of shear stress–induced NO release, modulates vascular permeability, and harbors a wide array of anticoagulant proteins. In a time course comparable with the loss of glycocalyx volume, we found loss of shear stress–induced NO release, increased vascular permeability, and activation of coagulation during hyperglycemic clamping in healthy volunteers. Our observation concerning the prevention of glycocalyx damage by antioxidant infusion confirms other research on the role of oxidative stress in hyperglycemia-induced vascular damage (6,39). Therefore, glycocalyx measurement may hold

promise as a tool for estimating cardiovascular risk and the impact of cardiovascular risk-lowering therapies in diabetic patients.

ACKNOWLEDGMENTS

H.V. has received a research fellowship from the Royal Netherlands Academy of Arts and Sciences (KNAW). J.J.P.K. is an established investigator of the Netherlands Heart Foundation (2000D039).

T.W.V.H., M.L., J.B.L.H., J.J.P.K., E.S.G.S., and H.V. designed the study. M.N., T.W.V.H., M.H.P.V.L., H.L.M., J.C.M.M., F.H., and M.C.L.G.G. performed the research. M.N., E.S.G.S., and H.V. analyzed the data and wrote the article. All authors critically reviewed the manuscript.

REFERENCES

1. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M: Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 339:229–234, 1998
2. Nathan DM, Lachin J, Cleary P, Orchard T, Brillon DJ, Backlund JY, O'Leary DH, Genuth S: Intensive diabetes therapy and carotid intima-media thickness in type 1 diabetes mellitus. *N Engl J Med* 348:2294–2303, 2003
3. Algenstaedt P, Schaefer C, Biermann T, Hamann A, Schwarzloh B, Greten H, Ruther W, Hansen-Algenstaedt N: Microvascular alterations in diabetic mice correlate with level of hyperglycaemia. *Diabetes* 52:542–549, 2003
4. Morigi M, Angioletti S, Imberti B, Donadelli R, Micheletti G, Figliuzzi M, Remuzzi A, Zoja C, Remuzzi G: Leukocyte-endothelial interaction is augmented by high glucose concentrations and hyperglycaemia in a NF-kB-dependent fashion. *J Clin Invest* 101:1905–1915, 1998
5. Du XL, Edelstein D, Dimmeler S, Ju Q, Sui C, Brownlee M: Hyperglycaemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site. *J Clin Invest* 108:1341–1348, 2001
6. Brownlee M: Biochemistry and molecular cell biology of diabetic complications. *Nature* 414:813–820, 2001
7. Nieuwdorp M, Meuwese MC, Vink H, Hoekstra JB, Kastelein JJ, Stroes ES: The endothelial glycocalyx: a potential barrier between health and vascular disease. *Curr Opin Lipidol* 16:507–511
8. Mulivor AW, Lipowsky HH: Role of glycocalyx in leukocyte-endothelial cell adhesion. *Am J Physiol* 283:H1282–H1291, 2002
9. Vink H, Constantinescu AA, Spaan JA: Oxidized lipoproteins degrade the endothelial surface layer: implications for platelet-endothelial cell adhesion. *Circulation* 101:1500–1502, 2000
10. Henry CB, Duling BR: Permeation of the luminal capillary glycocalyx is determined by hyaluronan. *Am J Physiol* 277:H508–H514, 1999
11. Weinbaum S, Zhang X, Han Y, Vink H, Cowin SC: Mechanotransduction and flow across the endothelial glycocalyx. *Proc Natl Acad Sci U S A* 100:7988–7995, 2003
12. Mochizuki S, Vink H, Hiramatsu O, Kajita T, Shigeto F, Spaan JA, Kajiya F: Role of hyaluronic acid in shear induced endothelium derived nitric oxide release. *Am J Physiol* 285:H722–H726, 2003
13. Florian JA, Kosky JR, Ainslie K, Pang Z, Dull RO, Tarbell JM: Heparan sulfate proteoglycan is a mechanosensor on endothelial cells. *Circ Res* 93:e136–e142, 2003
14. Constantinescu AA, Vink H, Spaan JA: Endothelial cell glycocalyx modulates immobilization of leukocytes at the endothelial surface. *Arterioscler Thromb Vasc Biol* 23:1541–1547, 2003
15. Thi MM, Tarbell JM, Weinbaum S, Spray DC: The role of the glycocalyx in reorganization of the actin cytoskeleton under fluid shear stress: a bumper-car model. *Proc Natl Acad Sci U S A* 101:16483–16488, 2004
16. Ceriello A, Giugliano D, Dello Russo P, Passariello N, Saccomanno F, Sgambato S: Glycosaminoglycans in human diabetes. *Diabetes Metab* 9:32–34, 1983
17. Ikegami-Kawai M, Okuda R, Nemoto T, Inada N, Takahashi T: Enhanced activity of serum and urinary hyaluronidases in streptozotocin-induced diabetic Wistar and GK rats. *Glycobiology* 14:65–72, 2004
18. Vink H, Duling BR: Identification of distinct luminal domains for macromolecules, erythrocytes, and leukocytes within mammalian capillaries. *Circ Res* 79:581–589, 1996
19. Vink H, Duling BR: Capillary endothelial surface layer selectively reduces plasma solute distribution volume. *Am J Physiol* 278:H285–H289, 2000
20. Orth VH, Rehm M, Thiel M, Kreimeier U, Haller M, Brechtelsbauer H, Finsterer U: First clinical implications of perioperative red cell volume measurement with a nonradioactive marker (sodium fluorescein). *Anesth Analg* 87:1234–1238, 1998
21. van Kreel BK, van Beek E, Spaanderman ME, Peeters LL: A new method for plasma volume measurements with unlabeled Dextran-70 instead of 125I-labeled albumin as an indicator. *Clin Chim Acta* 275:71–80, 1998
22. Williams SB, Goldfine AB, Timimi FK, Ting HH, Roddy MA, Simonson DC, Creager MA: Acute hyperglycemia attenuates endothelium-dependent vasodilation in humans in vivo. *Circulation* 97:1695–1701, 1998
23. Krentz AJ, Boyle PJ, Macdonald LM, Schade DS: Octreotide: a long-acting inhibitor of endogenous hormone secretion for human metabolic investigations. *Metabolism* 43:24–31, 1994
24. Witzig TE, Kvols LK, Moertel CG, Bowie EJ: Effect of the somatostatin analogue octreotide acetate on hemostasis in humans. *Mayo Clin Proc* 66:283–286, 1991
25. Vale JA, Proudfoot AT: Paracetamol (acetaminophen) poisoning. *Lancet* 346:547–552, 1995
26. Hijmering ML, Stroes ES, Pasterkamp G, Sierevogel M, Banga JD, Rabelink TJ: Variability of flow mediated dilation: consequences for clinical application. *Atherosclerosis* 157:369–373, 2001
27. Klitzman B, Duling BR: Microvascular hematocrit and red cell flow in resting and contracting striated muscle. *Am J Physiol* 237:H481–H490, 1979
28. Desjardins C, Duling BR: Microvessel hematocrit: measurement and implications for capillary oxygen transport. *Am J Physiol* 252:H494–H503, 1987
29. Wang A, Hascall VC: Hyaluronan structures synthesized by rat mesangial cells in response to hyperglycemia induce monocyte adhesion. *J Biol Chem* 279:10279–10285, 2004
30. Mulivor AW, Lipowsky HH: Inflammation- and ischemia-induced shedding of venular glycocalyx. *Am J Physiol* 286:H1672–H1680, 2004
31. Henry CB, Duling BR: TNF-alpha increases entry of macromolecules into luminal endothelial cell glycocalyx. *Am J Physiol* 279:H2815–H2823, 2000
32. Title LM, Cummings PM, Giddens K, Nassar BA: Oral glucose loading acutely attenuates endothelium-dependent vasodilation in healthy adults without diabetes: an effect prevented by vitamins C and E. *J Am Coll Cardiol* 36:2185–2191, 2000
33. Timimi FK, Ting HH, Haley EA, Roddy MA, Ganz P, Creager MA: Vitamin C improves endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 31:552–557, 1998
34. Cosentino F, Luscher TF: Tetrahydrobiopterin and endothelial function. *Eur Heart J* 19 (Suppl. G):G3–G8, 1998
35. Rao AK, Chouhan V, Chen X, Sun L, Boden G: Activation of the tissue factor pathway of blood coagulation during prolonged hyperglycemia in young healthy men. *Diabetes* 48:1156–1161, 1999
36. Esmon CT: Inflammation and thrombosis. *J Thromb Haemost* 1:1343–1348, 2003
37. Pearson MJ, Lipowsky HH: Effect of fibrinogen on leukocyte margination and adhesion in postcapillary venules. *Microcirculation* 11:295–306, 2004
38. Zuurbier CJ, Demirci C, Koeman A, Vink H, Ince C: Short-term hyperglycemia increases endothelial glycocalyx permeability and acutely decreases lineal density of capillaries with flowing RBCs. *J Appl Physiol* 99:1471–1476, 2005
39. Kurzelewski M, Czarnowska E, Beresewicz A: Superoxide- and nitric oxide-derived species mediate endothelial dysfunction, endothelial glycocalyx disruption, and enhanced neutrophil adhesion in the post-ischemic guinea-pig heart. *J Physiol Pharmacol* 56:163–178, 2005