Reduced Vascular NO Bioavailability in Diabetes Increases Platelet Activation In Vivo

Andreas Schäfer, Nicholas J. Alp, Shije Cai, Craig A. Lygate, Stefan Neubauer, Martin Eigenthaler, Johann Bauersachs, Keith M. Channon

Objective—Platelet activation is a feature of cardiovascular disease that is also characterized by endothelial dysfunction. The direct relationship between impaired endothelium-derived NO bioavailability and platelet activation remains unclear. We investigated whether acute inhibition of NO production modulates platelet activation in mice and whether specific rescue of endothelial function in diabetes modifies platelet activation.

Methods and Results—Intravenous injection of the NO synthase inhibitor N^6^-nitro-L-arginine methyl ester in wild-type (WT) mice significantly reduced platelet vasodilator-stimulated phosphoprotein (VASP) phosphorylation and increased platelet surface expression of P-selectin, CD40 ligand, and fibrinogen platelet binding, demonstrating that NO production exerts tonic inhibition of platelet activation in mice. Diabetes was induced by streptozotocin injection in WT or endothelial-targeted guanosine 5'-triphosphate cyclohydrolase I (GCH)-transgenic (GCH-Tg) mice protected from endothelial dysfunction in diabetes by sustained levels of tetrahydrobiopterin in vascular endothelium. Platelet VASP phosphorylation was significantly reduced in diabetic WT but not in diabetic GCH-Tg mice. P-selectin, CD40 ligand expression, and fibrinogen binding were increased in diabetic WT mice but remained unchanged compared with controls in endothelial-targeted GCH-Tg mice.

Conclusion—Platelet activation results from acute and chronic reduction in NO bioactivity. Rescue of platelet activation in diabetes by endothelial-specific restoration of NO production demonstrates that platelet function in vivo is principally regulated by endothelium-derived NO. (Arterioscler Thromb Vasc Biol. 2004;24:1720-1726.)

Key Words: diabetes • endothelial nitric oxide synthase • platelets • endothelial dysfunction

The endothelium plays a crucial role in control of vascular tone by releasing endothelium-derived autacoids, the most important of which is NO, generated by endothelial NO synthase (eNOS). NO also inhibits platelet activation, adhesion and aggregation; reduced NO bioactivity is associated with arterial thrombosis in animal models and in individuals with endothelial dysfunction.

The importance of NO in platelet function was shown by in vitro experiments such as inhibition of platelet aggregation by endothelial cells or NO donors. NO formation inhibition causes platelet activation, and exogenous NO can even reverse agonist-induced activation of platelet glycoprotein (GP) IIb/IIIa. The finding that platelets adhere to dysfunctional endothelium and that expression of potential adhesion molecules is enhanced under these conditions suggests that NO may modulate platelet activation in vascular disease states. We demonstrated recently that inhibition of systemic NO formation in healthy humans rapidly induces platelet activation, an effect that could be reversed by exogenous NO. Indeed, increased platelet activation is observed in diseases characterized by chronic endothelial dysfunction such as acute coronary syndromes, congestive heart failure, diabetes, and hypercholesterolemia. In patients with advanced atherosclerosis, impaired endothelium-dependent release of NO leads to reduced platelet cGMP formation. It remains uncertain whether endothelial-derived NO has a more important role in tonic suppression of platelet activation in vivo. In addition, the possibility that selective rescue of endothelial dysfunction in vascular disease states is sufficient to normalize platelet activation remains unexplored.

NO-dependent phosphorylation of the vasodilator-stimulated phosphoprotein (VASP) plays a pivotal inhibitory role in regulation of platelet activation. VASP phosphorylation correlates closely with inhibition of fibrinogen binding to the GP IIb/IIIa receptor. Increased bioavailability of endothelium-derived NO induces phosphorylation of VASP preferentially at its serine residues 239 (Ser239) and 157 (Ser157) by NO-dependent activation of soluble guanylyl cyclase and subsequent cGMP-mediated stimulation of cGMP-dependent kinases (cGKs). By modulating platelet actin filament interactions, VASP phosphorylation is able to...
affect initial sequences in platelet adhesion and activation.\textsuperscript{21-23} Additionally, NO induces cGMP-mediated and cGK-independent platelet inhibition.\textsuperscript{24}

Of several vascular disease states, patients with diabetes mellitus have particularly marked deficits in NO-mediated endothelial function and an increased risk of thrombosis and accelerated atherogenesis. Increased platelet reactivity has been suggested as a potential mechanism contributing to the accelerated atherosclerosis seen in diabetic patients by detrimental effects such as capillary microembolization, local progression of vascular lesions, and triggering of acute arterial thrombosis.\textsuperscript{12} Platelet activation leads to changes in shape, degranulation, and rapid surface expression of adhesion molecules such as P-selectin and CD40 ligand.\textsuperscript{25} P-selectin participates in platelet adhesion to leukocytes.\textsuperscript{26,27} CD40 ligand plays a crucial role in formation of arterial thrombi\textsuperscript{28} and endothelial inflammation\textsuperscript{29} that are enhanced in diabetes.\textsuperscript{30}

In diabetic mice, vascular endothelial dysfunction is associated with uncoupling of eNOS within the endothelium caused by oxidation of its essential cofactor tetrahydrobipterin (BH4), resulting in a specific loss of endothelial NO bioavailability.\textsuperscript{31} We found recently that targeted overexpression of the rate-limiting enzyme for BH4 biosynthesis (GTP-cyclohydrolase I; guanosine 5'-triphosphate cyclohydrolase I [GCH]) in endothelial cells in a transgenic (Tg) mouse strain (GCH-Tg) reduces eNOS uncoupling and restores NO-mediated endothelial function in diabetic mice, thus providing an in vivo model of selective rescue of endothelial NO bioactivity in diabetes.\textsuperscript{32}

In this study, we determined whether acute systemic withdrawal of NO by in vivo inhibition of NO can affect platelet activation in mice as proof of principle and how the selective prevention of endothelial dysfunction by maintaining endothelial NO bioavailability in GCH-Tg mice would influence platelet activation in diabetes.

**Methods**

**Animals**

All studies involving laboratory animals were conducted in accordance with the UK Home Office Animals (Scientific Procedures) Act of 1986. Mice were housed in temperature-controlled cages (20°C to 22°C) with a 12-hour light/dark cycle and given free access to water and formulated diets. GTP cyclohydrolase (GTPCH) Tg (GCH-Tg) mice, in which human GTPCH transgene overexpression is targeted to the vascular endothelium under the control of the mouse Tie-2 promoter, generated in a C57BL/6 background as described previously, have increased endothelial GTPCH mRNA expression and protein production, resulting in a 3- to 4-fold augmentation of vascular BH4 levels.\textsuperscript{32} All experiments were performed with heterozygote GCH-Tg mice. All experimental comparisons were made between male Tg and wild-type (WT) littermates aged 12 to 16 weeks.

**Diabetes Induction by Streptozotocin Injection**

A single high-dose streptozotocin (STZ) regimen was used to induce pancreatic islet cell destruction and persistent hyperglycemia, as described previously.\textsuperscript{33} STZ (10 mg/mL; Sigma) was freshly dissolved in sterile sodium citrate buffer (0.025 mol/L, pH 4.5) and used within 10 minutes. Mice received a single 160 mg/kg IP injection of STZ or citrate buffer (control). Blood glucose was monitored weekly using a 1-touch blood glucose meter (LifeScan). Hyperglycemia was defined as random blood glucose level >20 mmol/L for >3 weeks after injection. Whole blood samples were collected 4 weeks after STZ injection. Total glycohemoglobin content of blood samples (HbA1c) was measured using a commercially available kit obtained from Sigma.

**In Vivo Hemodynamics**

Invasive hemodynamics measurement was undertaken in animals under general anesthesia using 2% isoflurane added to 100% oxygen. A Millar catheter (1.4 French) was inserted into the right carotid artery and approached to the ascending aorta under continuous blood pressure recording. After correct placement and fixation of the catheter, the right jugular vein was dissected and cannulated with an intravenous line prepared for the injection of L-NAME (100 mg/kg body weight) was injected slowly via the intravenous line dissolved in a volume of 100 μL of isotonic saline solution. Hemodynamic changes were recorded continuously for an additional 60 minutes, after which a final blood sample for assessment of platelet activation was collected. Control animals were injected with 100 μL of isotonic saline and hemodynamics investigated to exclude a volume-dependent effect on hemodynamics.

**Platelet Preparation and Flow Cytometry**

General anesthesia was induced using the volatile anesthetic isoflurane. Under deep terminal anesthesia, the abdominal cavity was opened, and blood was taken by direct puncture of the inferior caval vein into a 1-mL syringe prepared with sodium citrate (3.8%). Whole blood was diluted with PBS (free of Ca\textsuperscript{2+} and Mg\textsuperscript{2+}, enriched with d-glucose [5.5 mmol/L] and 0.5% BSA) and incubated with a fluorescein isothiocyanate (FITC)-labeled anti-fibrinogen antibody (WAK-Chemie) for determination fibrinogen membrane binding, with anti-mouse FITC-conjugated anti-P-selectin (CD62P) antibody for surface expression of P-selectin, with anti-mouse FITC-conjugated anti-CD41 antibody for GP Ib expression, or with anti-mouse phosphatidylethanolamine (PE)-conjugated anti-CD154 for surface expression of CD40 ligand (all antibodies purchased from Becton Dickinson) for 10 minutes at room temperature. Control samples were incubated with unspecified FITC-PE-conjugated isotype IgG in parallel to arbitrarily adjust the unspecific binding to a mean fluorescence of 10, which is visually subtracted in the results graphs. Platelets were fixed with methanol-free formaldehyde (final concentration 1.5%) for 10 minutes and analyzed in a Becton Dickinson FACSCalibur at a low flow rate. The platelet population was identified on its forward- and side-scatter distribution, and 20,000 events were analyzed for mean fluorescence using CELLQuest software, version 3.1f. To assess platelet leukocyte adhesion, platelets within the leukocyte population were identified by the platelet-specific antigen CD41. The basal state of platelet activation was always assessed in unstimulated whole blood because preparation of platelet-rich plasma or washed platelets and subsequent in vitro stimulation would abrogate effects of endothelium-derived NO in vivo.

VASP phosphorylation was evaluated using FITC-labeled antibodies against phosphorylation of Ser\textsuperscript{239} (16C2 antibody [50 μg/mL]; Nano Tools) and Ser\textsuperscript{277} (5C6 antibody [50 μg/mL]; Nano Tools),\textsuperscript{13,19} after fixation of the blood samples by methanol-free formaldehyde (1.5%, 5 minutes). The samples were diluted with PBS and were allowed to permeabilize for 10 minutes after Triton X-100 (0.2% final) had been added. Samples were divided into 2, and 1 portion was stained at room temperature for 45 minutes with the respective FITC-labeled antibody (16C2 or 5C6), the other with the FITC-labeled antibody, preincubated with a saturating dose of a specific blocking phosphopeptide (incubation lasted ≥30 minutes at 4°C)\textsuperscript{34} to control for nonspecific binding, which was adjusted arbitrarily to a mean fluorescence of 10. All flow cytometry analyses were performed blinded to treatment and genotype.

**Statistics**

Values are means ± SEM. Statistical evaluation of platelet parameters was performed by Student t test or ANOVA followed by a Tukey test.
In Vivo Hemodynamics During NOS Inhibition

Because endothelial NO release is a physiologically important regulator of blood pressure in vivo, we recorded the changes in blood pressure to verify inhibition of NO release in vivo after NOS inhibition by infusion of the NOS inhibitor L-NAME (100 mg/kg; Figure 1) before analyzing platelet parameters, as demonstrated recently in humans. L-NAME infusion but not buffer infusion resulted in a rapid rise in arterial blood pressure, demonstrating inhibition of vascular NO production.

Platelet Activation After Acute NOS Inhibition

We used platelet VASP phosphorylation as a marker of platelet NO bioavailability after in vivo inhibition of NOS in healthy mice by intravenous L-NAME infusion, compared with control animals after saline infusion. Platelet VASP phosphorylation at both Ser157 (Figure 2A) and Ser239 (Figure 2B) was significantly reduced after L-NAME infusion. A representative flow-cytometry histogram displays VASP phosphorylation at Ser157 under control conditions (solid line) in relation to unspecific binding (filled gray) and nearly absence of fluorescence after systemic NOS inhibition (dotted line), indicating reduced NO-mediated signaling in platelets (Figure 2C). In parallel, NOS inhibition enhanced platelet activation as shown by significant increases in surface expression of P-selectin (Figure 3A) and CD40 ligand (Figure 3B) as markers of platelet degranulation, and by enhanced platelet-binding of fibrinogen on activated GP IIb/IIIa (Figure 3C), whereas the number of circulating platelet–leukocyte aggregates was not significantly increased after acute NOS inhibition (Figure 3D). Expression of total GP IIb/IIIa remained unaltered (data not shown). These data (as proof of principle) suggest that acute withdrawal of tonic systemic NO production in vivo rapidly reduces platelet NO bioactivity and significantly increases platelet activation.

Diabetes Induction

To investigate the mechanistic importance of chronic endothelial NO deficiency on platelet activation in vascular disease states, we induced diabetes in WT and GCH-Tg mice. GCH-Tg mice are protected from endothelial dysfunction in STZ-induced diabetes by an endothelium-specific overproduction of the essential eNOS cofactor BH4, which prevents eNOS uncoupling, as demonstrated by reduced superoxide generation and normalization of NO-mediated vasorelaxation. Accordingly, this model was used to investigate whether endothelial eNOS dysfunction alone could be responsible for platelet activation in diabetes and whether specific rescue of endothelial NOS function would normalize platelet activation. As shown in the Table, blood glucose and HbA1c and 4 weeks after injection of STZ were not different...
between WT and GCH-Tg mice but were significantly elevated compared with control animals that received buffer injection alone.

**VASP Phosphorylation in Platelets in Diabetes**

In platelets from diabetic mice, VASP phosphorylation at Ser157 (Figure 4A) and Ser239 (Figure 4B) was significantly reduced compared with platelets from nondiabetic controls. Injection of buffer instead of STZ was not associated with changes in platelet VASP phosphorylation. However, in diabetic animals with targeted GCH overexpression and preserved endothelial-specific NO production, VASP phosphorylation remained similar to healthy controls. A representative flow-cytometry histogram showing VASP phosphorylation at Ser157.

**Platelet Activation in Diabetes Mellitus**

Fibrinogen platelet binding was increased significantly in diabetic WT animals compared with healthy controls, as demonstrated in a rightward shift in the representative flow-cytometry histograms (Figure 5A) and in higher mean fluorescence (Figure 5B). As fibrinogen binds via the activated GP IIb/IIIa, we assessed the mean fluorescence for the integrin (CD41) within the platelet population, which did not differ between healthy and diabetic animals (data not shown).

Platelet degranulation markers were also increased in diabetic mice, as represented by the enhanced surface expression of the adhesion molecules P-selectin (CD62P; Figure 6A) and CD40 ligand (CD154; Figure 6B). These findings were associated with increased detection of circulating platelet–leukocyte aggregates in whole blood of diabetic animals, which is demonstrated by the higher fluorescence for the

**Biochemical Data From WT Mice and GCH-Tg Littermates 4 Weeks After Induction of Diabetes Using STZ (Diabetic) or After Buffer Injection (Control)**

<table>
<thead>
<tr>
<th></th>
<th>WT GCH-Tg</th>
<th>WT GCH-Tg</th>
<th>WT GCH-Tg</th>
<th>WT GCH-Tg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>7.6±0.5</td>
<td>29.1±0.7</td>
<td>7.1±0.3</td>
<td>24.3±1.8</td>
</tr>
<tr>
<td>HbA1 (%)</td>
<td>4.7±0.1</td>
<td>6.1±0.3†</td>
<td>4.7±0.2</td>
<td>6.9±0.5†</td>
</tr>
</tbody>
</table>

**Figure 4.** Platelet VASP phosphorylation at Ser157 (A) and Ser239 (B) in platelets from healthy (open bars) and diabetic (filled bars) WT or GTPCH-overexpressing Tg mice (GCH-Tg). Results are expressed as the mean fluorescence±SEM from 6 to 10 separate animals. **P<0.01 vs nondiabetic WT and diabetic GCH-Tg. C, Representative flow-cytometry histogram showing VASP phosphorylation at Ser157.

**Figure 5.** Platelet binding of fibrinogen in platelets from WT mice with and without diabetes compared with healthy and diabetic GCH-Tg mice shown as enhanced rightward shift of fibrinogen fluorescence in representative flow-cytometry histograms (A) and increased mean fluorescence for platelet-bound fibrinogen±SEM (n=6 to 10; B). **P<0.01 vs nondiabetic WT and diabetic GCH-Tg.

**Figure 6.** Surface expression of P-selectin (CD62P; A) and CD40 ligand (CD154; B) in healthy and diabetic WT and GCH-Tg mice, expressed as mean fluorescence±SEM. Platelet–leukocyte adhesion was determined by leukocyte mean fluorescence for platelet CD41 (C) and the ratio of CD41-positive leukocytes within all leukocytes (D; n=6 to 10). **P<0.01 vs nondiabetic WT and diabetic GCH-Tg.
platelet-specific antigen CD41 within the leukocyte population (Figure 6C). Accordingly, the ratio of leukocytes, which were CD41 positive, was increased in diabetes (Figure 6D). In contrast, platelets from diabetic animals with endothelium-targeted GCH overexpression showed no increases in fibrinogen binding, surface expression of P-selectin, or CD40 ligand, or in the appearance of platelet–leukocyte aggregates (Figure 6A through 6D). These data demonstrate (using the GCH-Tg as a tool to specifically assess effects of endothelium-derived NO in diabetes on platelets) that indeed endothelial NO critically determines platelet inhibition in vivo and that endothelial dysfunction plays a causative role for platelet activation in early diabetes mellitus.

**Discussion**

In this study, we described new findings that demonstrate an important role for endothelium-derived NO in regulating platelet activation in vivo. First, basal NO release tonically inhibits platelet activation in healthy animals; withdrawal of NO leads to rapid platelet activation. Second, increased platelet activation in diabetes, in association with chronically reduced NO bioavailability, is rescued by endothelium-specific preservation of eNOS function.

The relationship between reduced levels of endothelium-released NO and increased platelet activation is directly relevant to several vascular disease states, such as diabetes, hypertension, and hypercholesterolemia, that are characterized by endothelial dysfunction and enhanced platelet activity. The observation that platelets adhere to intact but dysfunctional endothelium and that expression of such potential adhesion molecules is enhanced under these conditions supports the hypothesis that endothelium-derived NO inhibits platelet activation. Our results in the GCH-Tg mice now clearly suggest a pivotal role for endothelium-derived NO in tonic inhibition of platelet activation under physiological conditions in vivo, and for loss of endothelium-derived NO in the pathogenesis of platelet activation in vascular disease states.

We used platelet VASP phosphorylation as a functionally important measure of platelet NO bioactivity. Conservation of platelet VASP phosphorylation in the GCH-Tg diabetes group is consistent with our previous observation of preserved vascular NO bioavailability and endothelial function in this Tg mouse model because VASP Ser157 and Ser239 phosphorylation reflect the bioactivity/integrity of platelet inhibitors/inhibitory pathways, including the predominant NO/cGMP pathway. Using specific antibodies, phosphorylation at Ser239 and Ser157 can be used as a sensitive monitor of defective NO/cGMP signaling, and reduced NO bioavailability in several pathophysiological states correlates with reduced VASP phosphorylation. VASP phosphorylation was significantly reduced in our model in WT diabetes, as it has been reported before for endothelial dysfunction in vascular tissues. These observations suggest that platelet VASP phosphorylation could be developed as a useful marker of endothelial (dys)function.

In addition to providing a marker of NO bioactivity, NO-dependent platelet VASP phosphorylation also has pivotal functional roles in platelet activation. Type I cGK-dependent VASP phosphorylation is known to affect its interaction with F-actin, its focal adhesion localization, and therefore is an important aspect of actin filament assembly/disassembly, a fundamental step of platelet activation. The importance of this signaling pathway is underlined by experimental studies demonstrating increased platelet adhesion and aggregation during cGK deficiency as well as increased levels of P-selectin expression and bound fibrinogen in VASP-deficient mice, which display enhanced platelet adhesion in vivo. Furthermore, aggregation in response to collagen is enhanced, whereas inhibition of platelet aggregation by cyclic nucleotides is impaired in platelets from these animals.

Accordingly, we found increased surface expression of P-selectin and CD40 ligand on unstimulated platelets in whole blood from diabetic WT mice. P-selectin can participate in platelet adhesion to the endothelium and is certainly responsible for platelet-leukocyte adhesion, which, in turn, was significantly increased in our study in diabetic animals. When VASP phosphorylation was preserved in diabetic GCH-Tg mice, expression of P-selectin and CD40 ligand was similar to that in healthy WT mice. This conforms with an earlier study reporting inhibition of P-selectin and CD40 ligand surface expression by cAMP/cGKs, activation of which can be monitored by VASP phosphorylation.

The extent of platelet activation observed in circulating platelets from diabetic WT mice was comparable to the magnitude of platelet activation induced by low doses of ADP (data not shown) and was certainly much lower than the activity that would be evoked by agonists such as thrombin or collagen. These results are in line with our recent findings in humans demonstrating similar platelet activation of unstimulated circulating platelets after systemic NOS inhibition. It might be possible (similar to vasodilating and vasoconstricting agents in vascular wall homeostasis) that NO might exert platelet-inhibiting effects under in vivo conditions, which might counteract known platelet agonists present in plasma. This could result in enhanced platelet activation during dysfunctional NO-mediated platelet inhibition in vivo. This is supported by studies even demonstrating enhanced adhesion of circulating platelets in vivo in cGK−/− or VASP−/− mice. It has been reported previously that a deficiency of NO supports and sustains platelet-mediated thrombotic responses in vivo.

We did not use agonist-induced stimulation of platelets in this study because preparation or stimulation of platelets in vitro would always abrogate the potential in vivo effects of endothelial NO, the effect of which was the main focus in this study. In vitro conditions would necessarily result in experimental conditions lacking endothelial NO and would unfortunately not be informative in determining the effect of endothelial NO and its bioavailability in vivo on platelet activation.

In most cardiovascular diseases, endothelial dysfunction is accompanied by endothelial activation and, hence, proinflammatory pathways could influence platelet activation in platelets rendered more susceptible to stimulation during reduced NO bioavailability. However, the identification of factors responsible for platelet activation of more susceptible plate-
lets under conditions of NO deficiency requires further study. Furthermore, lack of systemic NO release is accompanied by vasocostriction, which could exert more shear on circulating platelets and thereby potentially increase platelet activation.

Patients with diabetes mellitus have an increased risk of thrombosis and accelerated atherogenesis. The changes occurring in platelets from diabetic patients include enhanced expression/activation of GP IIb/IIIa and P-selectin, increased membrane binding of fibrinogen and plasma levels of serotonin, β-thromboglobulin, and soluble von Willebrand factor, as well as numbers of circulating monocyte–platelet aggregates.42 Furthermore, patients with diabetes exhibit an enhanced adhesion and aggregation of platelets on extracellular matrix.43 Patients with exaggerated platelet aggregation have an increased risk for restenosis after percutaneous transluminal coronary angioplasty.44 Prolonged hyperglycemia induces abnormal Ca²⁺ homeostasis similar to changes observed in platelets from diabetic patients.45 In animal studies, diabetes leads to enhanced susceptibility of platelets to agonists, fibrinogen binding, and platelet interaction with injured vessels, leading to increased atherosclerosis. Increasing levels of glucose have been determined as independent predictors of platelet-dependent thrombosis in patients with coronary artery disease.46 In platelets from diabetic patients, agonist-induced release of platelet-derived growth factor is enhanced.47 Even in metabolically stable, islet cell antibody-positive individuals, platelet activation markers (CD62, CD63, thrombospordin) were significantly increased.48

The impact of activated platelets on morbidity and mortality in diabetes is extremely relevant because enhanced platelet activation is an initial step in atherosclerosis and responsible for lesion progression, and excessive cardiovascular complications are associated with diabetes.50 The results of this study imply that endothelial dysfunction in diabetes not only affects the vascular environment of endothelial cells (e.g., enhanced vasoconstriction by smooth muscle cells, modulation of inflammatory processes in the vessel wall) but further locally contributes to changes within their luminal environment with possible major systemic interactions accounting for the enhanced cardiovascular morbidity and mortality in diabetic patients. The results from the CCH-Tg mice suggest that therapies to specifically target endothelial dysfunction in patients with diabetes or other vascular disease states should result in salutary effects on platelet activation, mediated through restoration of endothelium-derived NO.

Acknowledgments
Supported by the British Heart Foundation. A.S. was supported by the International Society for Heart Research-European Section/Servier Research Fellowship 2002 and the Deutsche Forschungsgemeinschaft DFG (SCHA 954/1–1).

References


Reduced Vascular NO Bioavailability in Diabetes Increases Platelet Activation In Vivo
Andreas Schäfer, Nicholas J. Alp, Shije Cai, Craig A. Lygate, Stefan Neubauer, Martin Eigenthaler, Johann Bauersachs and Keith M. Channon

Arterioscler Thromb Vasc Biol. 2004;24:1720-1726; originally published online July 8, 2004; doi: 10.1161/01.ATV.0000138072.76902.dd
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/24/9/1720

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/