**Plasma Nitroso Compounds Are Decreased in Patients With Endothelial Dysfunction**

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**OBJECTIVES**

We investigated whether plasma nitrosylated species (RXNOs) that mediate systemic nitric oxide (NO) bioactivity are depleted in individuals with cardiovascular risk factors and endothelial dysfunction.

**BACKGROUND**

Endothelium-derived NO acts not only as a regional messenger but exerts significant systemic effects via formation of circulating RXNOs delivering NO to sites of impaired production.

**METHODS**

Endothelial function was assessed in 68 patients with one to four major cardiovascular risk factors (RF) and 39 healthy control subjects (C) by measurement of flow-mediated dilation (FMD) of the brachial artery using high-resolution ultrasound. In parallel, plasma RXNOs were determined by reductive gas phase chemiluminescence.

**RESULTS**

Increasing numbers of risk factors were accompanied by a progressive decrease in FMD: 6.5 ± 0.4% (C); 4.7 ± 0.5% (one RF); 2.8 ± 0.4% (two RF); 2.2 ± 0.4% (three RF); and 1.0 ± 0.3% (four RF). Progressively impaired vascular function was associated with a concomitant decrease in plasma RXNOs (p < 0.01): 39 ± 2 nmol/l (C); 30 ± 2 nmol/l (one RF); 24 ± 3 nmol/l (two RF); 22 ± 3 nmol/l (three RF); and 15 ± 2 nmol/l (four RF), with univariate correlation between FMD and RXNO (r = 0.41, p < 0.001). In a multivariate regression model, RXNO was an independent predictor of endothelial function.

**CONCLUSIONS**

Endothelial dysfunction in patients with cardiovascular risk factors is associated with decreased levels of circulating RXNOs. Plasma RXNOs may be diagnostically useful markers of NO bioavailability and a surrogate index of endothelial function. Whether the observed decrease in concentration reflects impaired NO formation, accelerated decomposition, and/or consumption of RXNOs and whether these processes play a causal role in the pathophysiology of arteriosclerosis remain to be investigated. (J Am Coll Cardiol 2006;47:573–9)

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Manuscript received May 5, 2005; revised manuscript received June 3, 2005, accepted June 27, 2005.

Arteriosclerosis is the major cause for chronic vascular diseases such as coronary artery disease, cerebrovascular disease, and peripheral arterial occlusive disease. The key event in the pathogenesis of arteriosclerosis is believed to be dysfunction of the endothelium and disruption of endothelial homeostasis, predisposing blood vessels to vasoconstriction, inflammation, leukocyte adhesion, thrombosis, and proliferation of vascular smooth muscle cells.

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Endothelium-derived nitric oxide (NO) is an essential short-lived signaling molecule important for vascular homeostasis. Decreased NO bioactivity leads to accelerated atherogenesis. Until recently, it was believed that the bioactivity of NO is limited to close temporal and spatial proximity of the endothelium and that NO is a mere autocrine/paracrine effector (i.e., that it can only travel short distances in the bloodstream (1) before it becomes inactivated). Recent studies challenge this concept by suggesting that free NO is in equilibrium with a pool of various NO-containing compounds in blood that have a bioactivity that resembles that of authentic NO. Inhaled and intravenously applied exogenous NO is transported and delivered along the vascular tree to dilate distal conduit arteries and the microvasculature (2,3). While we have recently reported that this “circulating NO pool” can be modulated by dietary intervention (4), the chemical identity of those species is currently unclear. Initially, the circulating pool was believed to be mainly comprised of nitrosothiols, in particular S-nitrosoalbumin (>80%), and to a lesser extent S-nitrosohemoglobin, S-nitrosoglutathione, and S-nitrosocysteine (5). Consistent with this view, intravenous application of authentic S-nitrosothiols leads to vasodilation (2), inhibition of platelet activation (6), abolition of carotid embolization (7), and protection from ischemia/reperfusion injury (8). More recently, however, N-nitroso proteins and iron-nitrosyl complexes (9,10), as well as nitrite (11,12) and potentially nitrated lipids (13), have also been implicated to serve as plasma NO stores.

The measurement of flow-mediated dilation (FMD) of the brachial artery (BA) as a noninvasive endothelial func-
tion test in humans was introduced in 1992 by Celermajer et al. (14), and this approach has now been used by numerous groups throughout the world to monitor endothelial function. This ultrasonographic method quantifies the dilution of conduit arteries in response to physiologically relevant increases in laminar shear stress induced by ischemic dilation of the downstream microvasculature. Increases in shear stress (i.e., the tangential force exerted by the flow of blood over the surface of the endothelium) lead to a rapid activation of endothelial NO synthase (eNOS) with consecutive increases in NO formation. Accordingly, FMD is largely abolished after NOS inhibition, and, therefore, provides a valuable "read-out" of local vascular NO availability.

Using this approach, it has been shown that the conditions associated with the major cardiovascular risk factors have an additive negative impact on endothelial function that is observable well before clinically apparent vascular complications. A depletion of the "circulating NO pool" under these conditions may contribute to the progression of arteriosclerosis and trigger or aggravate cardiovascular emergencies. Whether the concentration of circulating NO species is indeed decreased in individuals with endothelial dysfunction, however, is currently unknown. We, therefore, sought to investigate the hypothesis that the circulating sum of NO species (RXNOs) are progressively depleted in individuals with increasing numbers of major cardiovascular risk factors and that a lower concentration of this bioactive NO pool in human plasma correlates with the degree of endothelial dysfunction as measured by FMD.

**METHODS**

**Study subjects.** We studied 68 patients with one to four major cardiovascular risk factors and 39 healthy subjects without clinical evidence for other cardiovascular risk factors recruited from the outpatient clinic of the department of internal medicine. Patients with reduced left ventricular function or signs of heart failure, valvular disease, and arrhythmogenic disorders were excluded. Major cardiovascular risk factors were hypertension, hypercholesterolemia, smoking, or diabetes mellitus. Hypertension was defined by a systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg (15), or current antihypertensive medication. According to American Diabetes Association guidelines (16), diabetes mellitus was defined by glucose levels in plasma >126 mg/dl (fasting), >200 mg/dl (2 h after oral glucose tolerance), >300 mg/dl (any time in combination with diabetic symptoms), or current antidiabetic medication.

Hypercholesterolemia was defined by total cholesterol levels >240 mg/dl, low-density lipoprotein cholesterol levels >160 mg/dl, or cholesterol-lowering medication (17). Coronary artery disease was diagnosed by coronary angiography. The clinical characteristics are summarized in Table 1. Patients were asked to refrain from smoking and stay fasted from 8:00 PM of the night before and until completion of investigations. Current medication (Table 2) was discontinued for >18 h before and during the investigations (i.e., "night" and "morning" medication was withheld). Informed consent was obtained from all study subjects before enrollment. The study protocol was approved by the local ethics committee of the Heinrich-Heine University of Duesseldorf.

**NO species, nitrite, and nitrate in plasma.** Before vascular measurements were performed, blood was drawn from...

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**Table 1. Clinical Characteristics of the Study Population**

<table>
<thead>
<tr>
<th>No. of Cardiovascular Risk Factors</th>
<th>0 (Control)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>p Value (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>n = 39</td>
<td>n = 31</td>
<td>n = 10</td>
<td>n = 14</td>
<td>n = 13</td>
<td>&lt;0.001</td>
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<td>Gender, M/F</td>
<td>19/20</td>
<td>16/15</td>
<td>7/3</td>
<td>11/3</td>
<td>12/1</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.1 ± 0.4</td>
<td>24.0 ± 0.5</td>
<td>27.3 ± 0.9*</td>
<td>27.0 ± 0.7*</td>
<td>28.6 ± 1.1*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>n = 0</td>
<td>n = 4</td>
<td>n = 4</td>
<td>n = 13</td>
<td>n = 13</td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>n = 0</td>
<td>n = 9</td>
<td>n = 7</td>
<td>n = 13</td>
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<td></td>
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<tr>
<td>Diabetes mellitus</td>
<td>n = 0</td>
<td>n = 1</td>
<td>n = 1</td>
<td>n = 4</td>
<td>n = 11</td>
<td></td>
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<tr>
<td>Smoker</td>
<td>n = 0</td>
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<td>n = 6</td>
<td>n = 12</td>
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<tr>
<td>Coronary artery disease</td>
<td>n = 1</td>
<td>n = 2</td>
<td>n = 4</td>
<td>n = 13</td>
<td>n = 13</td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>93 ± 1</td>
<td>90 ± 2</td>
<td>96 ± 3</td>
<td>101 ± 2*</td>
<td>100 ± 3*</td>
<td>0.002</td>
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<tr>
<td>Total cholesterol, mg/dl</td>
<td>200 ± 4</td>
<td>209 ± 8</td>
<td>214 ± 16</td>
<td>245 ± 44</td>
<td>195 ± 2</td>
<td>0.289</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>137 ± 4</td>
<td>147 ± 7</td>
<td>150 ± 13</td>
<td>144 ± 10</td>
<td>135 ± 12</td>
<td>0.587</td>
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<tr>
<td>HDL cholesterol, mg/dl</td>
<td>68 ± 2</td>
<td>68 ± 4</td>
<td>57 ± 6</td>
<td>48 ± 5*</td>
<td>56 ± 2</td>
<td>0.002</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>90 ± 6</td>
<td>110 ± 91</td>
<td>146 ± 18</td>
<td>432 ± 261*</td>
<td>207 ± 59</td>
<td>0.021</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>90 ± 1</td>
<td>90 ± 2</td>
<td>97 ± 7</td>
<td>111 ± 9*</td>
<td>136 ± 15*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pack-yrs</td>
<td>0†</td>
<td>8.9 ± 2.0*</td>
<td>13.2 ± 4.3*</td>
<td>29.8 ± 4.1*</td>
<td>38.9 ± 4.6*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SE. p values from analysis of variance (ANOVA). *p < 0.05 vs. control; †p < 0.05 vs. one risk factor.

BMI = body mass index; HDL = high-density lipoprotein; LDL = low-density lipoprotein.
the antecubital vein. All measurements were performed after overnight fasting of more than 12 h between 8:00 and 9:00 AM. Plasma levels of nitros(y)lated NO species (RXNO: the sum of S-nitrosothiols, N-nitrosamines, iron-nitrosyl species) and nitrite were determined using a triiodide/ozone-based chemiluminescence assay, essentially as described (18,19). A high reproducibility of RXNO measurements has been shown previously for standards (19). In addition, we have performed repeated measurements of plasma RXNOs on two separate days at the same time of the day in fasted healthy volunteers (n = 20) and found an intra-subject variation (i.e., averaged deviation between measurements of 1.8 ± 9%). Nitrate was quantified after enzymatic reduction to nitrite by nitrate reductase using flow-injection analysis (20). All analyses were performed by independent investigators blinded to the clinical history of study subjects.

Endothelium-dependent and endothelium-independent vasodilation. Endothelium-dependent dilation of the BA was measured noninvasively by high-resolution ultrasound (SONOS 5500, Agilent Technologies, Palo Alto, California, with a 15-MHz linear-array transducer) using standard techniques (21). Briefly, baseline data for diameter and blood-flow velocity of the BA were quantified after 10 min of supine rest in an air-conditioned room (21°C) 1 to 2 cm above the elbow. Then a blood pressure cuff was placed around the forearm distal to the cubital fossae and inflated above the elbow. Then a blood pressure cuff was placed (hyperemic blood flow) as well as 60, 75, 90, and 120 s later. Maximal BA diameter observed during this time period was used to calculate FMD. Endothelium-independent dilation were expressed as a percentage change from baseline. To estimate the relative proportion of FMD compared to the maximally achievable diameter after GTN, the FMD/GTN ratio was calculated and expressed as percentage for each individual. Blood flow was calculated by multiplication of cross-sectional area and mean velocity at each time point.

Statistical analysis. Results are expressed as means ± SE. Comparisons between groups were analyzed by analysis of variance (ANOVA) and, if significant, consecutive post-hoc test (Bonferroni). P values from Bonferroni post-hoc tests were corrected for 10 pairwise comparisons. Linear relationships between continuous variables were calculated using Pearson’s r. A multivariate regression analysis was performed, to estimate the predictive value of plasma RXNO concentrations to explain the variability of vascular function as a surrogate for cardiovascular risk. Established parameters known to affect FMD were also included in the model. Standardized coefficients were calculated as a measure for the relative predictive value. Statistical significance was assumed if a null hypothesis could be rejected at p < 0.05. All analyses were performed with SPSS 11.0.1 (SPSS Inc., Chicago, Illinois).

RESULTS

Effect of cardiovascular risk factors on vascular parameters. Endothelial function measured by FMD was significantly impaired in individuals with increasing numbers of cardiovascular risk factors (one risk factor: 4.7 ± 0.4%; two risk factors: 2.8 ± 0.4%; three risk factors: 2.2 ± 0.4%; four risk factors: 1.0 ± 0.3%) compared to control subjects (6.5 ± 0.4%; p < 0.005 Bonferroni; Fig. 1A), irrespective of the nature of risk for cardiovascular disease. Flow-mediated dilation in individuals with one risk factor was significantly greater than in those with three and four risk factors (p = 0.003, Bonferroni). Endothelium-independent dilation in response to GTN was similarly impaired: 14.5 ± 1.0% (zero risk factors); 12.3 ± 1.0% (one risk factor); 10.6 ± 1.3% (two risk factors); 9.1 ± 1.9% (three risk factors); 6.8 ± 1.0% (four risk factors) (ANOVA: p < 0.05). However, the
significantly lowered FMD/GTN ratio indicates preferential endothelial dysfunction (Fig. 1B). The baseline diameter was significantly increased in the groups with three (5.1 ± 0.2 mm) and four risk factors (5.1 ± 0.2 mm) compared to subjects with zero to two risk factors (control: 4.0 ± 0.1 mm; one risk factor: 4.3 ± 0.1 mm; two risk factors: 4.7 ± 0.3 mm; \( p < 0.001 \) Bonferroni). No significant differences were seen in terms of blood flow at baseline, during hyperemia, or Doppler flow reserve (data not shown).

**Effect of cardiovascular risk factors on NO-related metabolites in plasma.** In individuals without risk factors, RXNO plasma concentration amounted to 39 ± 2 nmol/l. Increasing numbers of risk factors were associated with a significant reduction in plasma RXNO levels: 30 ± 2 nmol/l (one risk factor); 24 ± 3 nmol/l (two risk factors); 22 ± 3 nmol/l (three risk factors); and 16 ± 2 nmol/l (four risk factors) (\( p < 0.001 \) Bonferroni; Fig. 1C). Also, RXNO was significantly different in the groups with one and four risk factors (\( p = 0.003 \) Bonferroni). Plasma nitrite concentrations followed a similar trend without reaching statistical significance: 142 ± 13 (control); 125 ± 13 (one risk factor); 131 ± 23 (two risk factors); 118 ± 17 (three risk factors); and 106 ± 19 (four risk factors) (ANOVA: \( p = 0.821 \)). Nitrate concentrations were not significantly different between groups: 21 ± 3 \( \mu \)mol/l (control); 23 ± 3 \( \mu \)mol/l (one risk factor); 26 ± 5 \( \mu \)mol/l (two risk factors); 32 ± 7 \( \mu \)mol/l (three risk factors); and 23 ± 4 \( \mu \)mol/l (four risk factors) (ANOVA: \( p = 0.800 \)).

**Endothelial function correlates with circulating NO pool.** There was a highly significant correlation between FMD and plasma RXNO concentration (\( r = 0.41; p < 0.001 \); Fig. 2) suggesting an association between endothelial dysfunction and circulating NO pool.

**RXNOs as an independent predictor of endothelial dysfunction.** In order to establish RXNO concentration as an independent predictor of endothelial function, we performed a multivariate linear regression analysis including baseline characteristics known to affect vascular function (age, gender, body mass index, total cholesterol, plasma glucose, mean arterial blood pressure, pack years, diameter of BA, nitrite) and current medication with angiotensin-converting enzyme inhibitors and statins. As shown in Table 3, the independent predictor variables for FMD were gender, age, RXNO concentration, and BA diameter accounting for 53% of the total variability of FMD (adjusted \( R^2 = 0.526; p < 0.001 \)). The magnitudes of standardized coefficients for the independent predictors were in the same range as gender and age suggesting a comparable predictive value.

**DISCUSSION**

The key findings of the present study are that: 1) the plasma pool of RXNOs is depleted in patients with cardiovascular risk factors; and 2) RXNO concentration in plasma is a significant independent predictor of endothelial function.
Table 3. Multivariate Linear Regression Analysis

<table>
<thead>
<tr>
<th></th>
<th>Standardized Coefficient</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>0.224</td>
<td>0.029</td>
</tr>
<tr>
<td>Age</td>
<td>-0.189</td>
<td>0.045</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.039</td>
<td>0.706</td>
</tr>
<tr>
<td>Mean arterial blood pressure</td>
<td>0.091</td>
<td>0.267</td>
</tr>
<tr>
<td>Pack-ys</td>
<td>-0.010</td>
<td>0.920</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>-0.048</td>
<td>0.552</td>
</tr>
<tr>
<td>Plasma glucose</td>
<td>-0.161</td>
<td>0.052</td>
</tr>
<tr>
<td>RXNO</td>
<td>0.163</td>
<td>0.044</td>
</tr>
<tr>
<td>Nitrite</td>
<td>0.093</td>
<td>0.212</td>
</tr>
<tr>
<td>Brachial artery diameter</td>
<td>-0.377</td>
<td>0.001</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>-0.189</td>
<td>0.077</td>
</tr>
<tr>
<td>Statins</td>
<td>-0.066</td>
<td>0.527</td>
</tr>
<tr>
<td>Adjusted R²</td>
<td>0.526</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

ACE = angiotensin-converting enzyme; ANOVA = analysis of variance; BMI = body mass index; FMD = flow-mediated dilation; RXNO = sum of circulating nitric oxide species.

risk factors; and that 2) RXNO is an independent predictor of endothelial function in a multivariate regression model.

The earliest studies of endothelial control of vasomotion used quantitative coronary angiography to examine the vasomotor response of the epicardial coronary artery during infusion of acetylcholine (22) or increased blood flow (23). In healthy individuals, the endothelium responds to these stimuli by releasing vasodilator factors, in particular NO. Patients with angiographically documented coronary artery disease display impaired FMD and a vasoconstrictor response to acetylcholine, which likely reflects loss of NO and unopposed muscarinic receptor activation at the level of the vascular smooth muscle. Notably, studies suggest that endothelial function detected noninvasively in the BA correlates with function in conduit coronary arteries (24).

As in the coronary circulation, endothelial function in the brachial circulation is impaired in the setting of traditional and novel risk factors and responds to interventions known to reduce cardiovascular disease risk (25). Traditional cardiovascular risk factors as diverse as smoking, aging (26), hypercholesterolemia, hypertension, hyperglycemia, and a family history of premature atherosclerotic disease are all associated with attenuation/loss of endothelium-dependent vasodilation in both adults and children. More recently recognized risk factors such as obesity, elevated C-reactive protein, and chronic systemic infection are also associated with endothelial dysfunction. Corroborating several studies by Celermajer et al. (14) and other groups, we observed an inverse association between the cumulative number of cardiovascular risk factors and FMD of the BA.

Although case-control studies indicate an association between endothelial dysfunction and acute coronary syndromes (27), more convincing evidence for a pathogenic role of the former is provided by studies demonstrating that endothelial function identifies patients at increased risk for future events. Numerous published studies involving >2,000 patients with atherosclerosis have proven the prognostic value of endothelial vasomotor dysfunction (25). These studies strongly and consistently demonstrate that endothelial dysfunction identifies patients who have increased risk for cardiovascular events in the short and long run. Primary and secondary preventive therapies (e.g., exercise therapy, angiotensin-converting enzyme inhibitors, and statins) are believed to mediate their positive prognostic effect in large part by increasing endothelial function. In our study, we did not observe significant contributions of body mass index or current medication with angiotensin-converting enzyme inhibitors or statins to the variability of endothelial function in our multivariate regression model. This may either be due to the small n-number or the significantly greater predictive value of other included parameters. Thus, endothelial dysfunction appears to be a systemic process that can be identified in different vascular beds, including those remote from the coronary and cerebral circulation where the majority of actual events occur.

The present study is the first to report that RXNO levels in plasma are lower in individuals with one or more cardiovascular risk factors compared to controls. The importance of RXNOs results from the very similar biological actions exhibited by both NO and RXNOs in vivo as well as in vitro (28). Cannon et al. (3) have provided the first evidence for the intravascular delivery of bioactive NO species in humans. Inhaled NO restored blood flow and vascular resistance of the forearm during regional inhibition of NO synthesis. Intravenous infusion of NO in healthy volunteers increased plasma RXNOs and induced systemic hemodynamic effects at the level of both conduit and resistance vessels, as reflected by dilator responses in the BA and forearm microvasculature. Infusion of S-nitrosoglutathione exerted similar effects, and the observed changes in RXNO concentrations correlated with the vasodilator effects exerted by NO and S-nitrosoglutathione (2). Furthermore, RXNOs inhibit platelet aggregation (29) and abolish embolization from carotid plaques (30).

The RXNO levels reported in the literature range from the low nanomolar up to the micromolar range. This may be due to differences in the methodology applied to measure S-nitrosothiols and N-nitrosamines, sample preparation and handling, choice of standards, and detection methods (5,9,10,19,31,32). In view of the fact that the chemical identity of most of these species is currently unknown, we intentionally opted to measure the sum of all nitroso and nitrosyl species detectable under these conditions attempting a group-specific analysis as opposed to quantification of select species of unclear physiological role (19). Regardless of the absolute levels of RXNOs, the significant difference between the patient groups in relation to the impairment of endothelial function remains (31). Nevertheless, future studies are needed to identify relevant individual bioactive compounds comprising RXNOs.

The decreased pool of circulating NO with increasing cardiovascular risk load may be secondary to endothelial...
dysfunction reflected by the impaired FMD. This is underscored by the univariate correlation between FMD and the circulating NO pool and the multivariate analysis with RXNO being a significant predictor for FMD independent of age, gender, body mass index, blood pressure, cholesterol, glucose, baseline diameter of the BA, pack years, current statin and angiotensin-converting enzyme inhibitor therapy with comparable magnitude as gender and age. Corroborating our previous findings, the baseline diameter was also a significant predictor (33). Also, cigarette smoke contains large amounts of nitrogen oxides (34); the subgroup of smokers without other cardiovascular risk factors showed decreased RXNOs and FMD. Potentially, the reduced circulating NO pool may accelerate atherogenesis and predispose individuals to clinical arteriosclerotic syndromes characterized by impaired regional NO production, such as peripheral and coronary artery disease. Whether decreased levels of RXNOs are a consequence of reduced endogenous NO synthesis and/or bioavailability or that of an accelerated breakdown and/or NO consumption is currently unclear. In addition to a diminished NO production, alterations in NOS expression, co-factor, and/or substrate availability may also contribute directly to oxidative stress by producing superoxide as a consequence of uncoupling of NOS. Thus, although the sources of oxidative stress may differ and several different enzymatic and biochemical mechanisms can disrupt normal NO signaling, a central problem appears to be a shift in NO/nitroso-redox balance away from “physiologic” heme nitrosylation and S-nitrosation to one of enhanced nitrosation/nitration of protein targets under conditions of oxidative stress. Whether plasma RXNO concentration represents a valid surrogate marker for cardiovascular risk burden or oxidative stress and qualifies as a prognostic factor cannot be concluded from the present study and remains to be investigated.

In newer publications, nitrite, one of the major oxidative metabolites of NO, was implicated to be both an indicator for NOS activity (35) and a circulating NO donor that may selectively donate NO to hypoxic vascular beds (11). Thus, apart from RXNOs, nitrite within the blood and tissues (11,36,37) may evolve as an important contributor to the circulating pool of bioactive NO. Although in the present study no significant differences in mean plasma nitrite levels were observed between individual groups, there was clearly an inverse relationship between nitrite levels and the number of cardiovascular risk factors. The fact that RXNOs, but not nitrite, predicts endothelial dysfunction in the multivariate regression model in this relatively small sample size may be explained by several differences in biochemical features of both compounds. The short half-life of plasma nitrite in blood, which is a consequence of its rapid transition (38) into red blood cells and surrounding tissues (19), contributes to its usefulness to index acute changes in eNOS activity (35). Although the absolute levels are conserved throughout many mammalian species (36), the intraindividual variability still poses a problem because diet, NO donor drugs, renal function, gut bacteria (39), and other NOS-independent confounders may influence baseline levels and thus require relatively large n-values to detect clear differences between patient populations. Because of its longer half-life and apparent robustness against influence by NOS-independent dietary confounders such as nitrate (40), RXNOs may be a more suitable diagnostic marker for long-term changes in eNOS activity. Nevertheless, new analytical methods capable of differentiating between individual RXNO components and an identification of the physiological roles of these species are necessary. Despite these obstacles, a composite view and analysis of the constituents of the circulating NO pool is a promising first step toward the development of new biochemical approaches to assess endothelial dysfunction.

Conclusions. From the data presented herein, we conclude that endothelial dysfunction is associated with a depletion of circulating nitroso/nitrosyl species in plasma, which is likely to contribute to the increased risk for major cardiovascular events in individuals with endothelial dysfunction. Measuring circulating nitroso/nitrosyl species may help in identifying individuals at risk and serve as a therapeutic surrogate marker in the future. Further studies are needed to establish whether the plasmatic RXNO pool represents a valuable parameter that allows optimal dose titration of therapeutic agents aimed at targeting endothelial dysfunction.

Acknowledgment

The authors wish to thank Gabi Schoder for her technical assistance.

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REFERENCES


