EDITORIAL COMMENT
Nitric Oxide Metabolites and Cardiovascular Disease
Markers, Mediators, or Both?*
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The chemical reactivity of the free radical gas nitric oxide (NO) promotes rapid formation of other nitrogen oxides, most of which have been considered biologically inert. Therefore, the general view has been that NO acts in a paracrine manner, exerting effects only in the close vicinity of its site of production. This view is now rapidly changing because it is becoming clear that many of NO's reaction products can convert back into bioactive NO. One example that has received great attention lately is the nitrite ion (NO$_2^-$), a product formed rapidly by oxidation of NO. It is now clear that several pathways exist for reduction of nitrite back to NO, and nitrite is in fact to be considered a major storage pool of NO in blood and tissues (1–3). Another example is the formation of s-nitrosothiols, which may act as stable carriers of NO.

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This new information adds a new dimension to measurements of NO reaction products in health and disease. Instead of being used merely as an index of historical NO formation, e.g., by the endothelium, they could actively be playing a role in the pathophysiological processes of cardiovascular disease. In this issue of the Journal, Heiss et al. (4) report on measuring a variety of NO-related metabolites in patients with endothelial dysfunction. The investigators report that the levels of nitrosylated compounds in plasma are markedly reduced in the patients, and this decrease is correlated to the severity of endothelial dysfunction as assessed by measurements of flow-mediated dilation of the brachial artery. The investigators therefore suggest that these NO related species could be used as markers of endothelial function.

One of the NO-related metabolites, nitrite, has previously been reported to be a good marker of endothelial NO production. Indeed, the investigators of the present article have estimated that as much as 70% of plasma nitrite originates from endothelial nitric oxide synthase (eNOS) in the endothelium (5). Therefore, it was surprising that nitrite was not significantly decreased in the present study. This may be related to the rather small sample size, but another likely explanation is the existsents of other eNOS-independent sources of plasma nitrite (see later text).

As the investigators point out, we do not know whether the low nitroso compound levels are caused by a decreased endothelial NO formation or by an accelerated consumption of these species. A major remaining question is whether the decreased nitroso compound levels are playing a part in the pathologic process, and this question could not be answered in the present study. Such future studies could involve pre-treating animals with physiological levels of the individual nitroso compounds and then looking at protective effects, e.g., in ischemia reperfusion models, or even study of physiological effects of nitroso compound supplementation to patients with low levels using a similar test array as the one used here. In fact, for one of the NO-related metabolites, nitrite, this has been recently done and the results are intriguing, showing powerful protective effects of nitrite in animal models of ischemia-reperfusion injury (6–8). In these studies, it is suggested that nitrite acts via formation of NO, but there is also a possibility that nitrite can modulate physiological processes via other pathways, for example, through direct s-nitrosylation of thiol-containing proteins.

Organic nitrates, for example nitroglycerine, are thought to act via generation of NO in smooth muscle cells, but the exact mechanism of action is still a matter of debate despite intense research for more than a century. Interestingly, recent studies now indicate that the nitrite ion is an obligate intermediate in the formation of NO. The further reduction of nitrite to NO in the cell may follow one of several pathways (1). This again indicates that nitroso compounds are not just inert waste products of NO metabolism but may in fact be key players in biological processes.

An important next task will be to better define the source of the various nitroso compounds in the circulation. Likely, the levels are determined not only by endogenous NO from NO synthases but also by dietary factors, medications (e.g., the use of nitrovasodilators), and smoking habits (cigarette smoke contains huge amounts of nitrogen oxides). One of the most intriguing possibilities of nitroso compounds in relation to cardiovascular disease is the fact that the systemic levels are greatly influenced by the diet (Fig. 1). As an example, vegetables (especially green leafy vegetables) contain great amounts of nitrate (NO$_3^-$). Interestingly, ingestion of nitrate in an amount corresponding to about 300 g spinach results in an almost four-fold increase in plasma levels of nitrite (9). Conversion of nitrate to nitrite involves enterosalivary circulation of nitrate and reduction to nitrite in the oral cavity by commensal bacteria (10,11). Nitrite then enters the circulation after swallowing of saliva. The obtained systemic levels of nitrite are in the same range as those that have potent cardioprotective effects in animal models, in which nitrite was given systemically before induction of cardiac ischemia. This implies that systemic levels of potentially cardioprotective nitroso compounds can be influenced by the diet and offers a highly intriguing new
explanation for the cardioprotective effects of a vegetarian diet (10). Naturally, all of these assumptions need to be tested in controlled clinical studies.

We now know from the interesting study by Heiss et al. (4) that plasma nitroso compound levels are decreased in patients with endothelial dysfunction, a finding that could be of diagnostic interest. The next critical step will be to elucidate the true role of plasma nitroso compounds in the pathophysiology of cardiovascular disorders, which may very well expand the concept to also involve therapeutic opportunities.

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