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Simple and Reliable Measurement of Nitric Oxide Metabolites in Plasma

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Featured article: Moshage H, Kok B, Huizenga JR, Jansen PLM. Nitrite and nitrate determinations in plasma: a critical evaluation. Clin Chem 1995;41: 892–6.[2]

The history of nitric oxide (NO•) is a remarkable one. In the early 1980s it was established that nitric oxide was produced in the human body. Shortly afterward it became clear that nitric oxide had important functions in the regulation of vascular tone, and it was demonstrated that nitric oxide was identical to endothelium-derived relaxing factor, a factor derived from endothelial cells that induced relaxation of smooth muscle cells. Nitric oxide was selected as the 1992 Molecule of the Year by the journal *Science*, and the 1998 Nobel Prize for Medicine or Physiology was awarded to Louis Ignarro, Ferid Murad, and Robert Furchgott, the founders of nitric oxide research. Since its discovery, the gaseous radical nitric oxide has elicited much attention from the scientific community, and nitric oxide has been implicated in many diverse processes ranging from the regulation of vascular tone and male erectile function to neurotransmission and microbiocidal activity.

Soon after the discovery that nitric oxide was produced in the body, nitric oxide was reported to be the product of the enzyme nitric oxide synthase (NOS), of which 3 isoforms exist: neuronal NOS, inducible NOS, and endothelial NOS. Neuronal NOS and endothelial NOS, constitutively expressed calcium/calmodulin-dependent isoforms, demonstrate that nitric oxide plays an important role in normal homeostasis. On the other hand, inducible NOS is the inducible isoform expressed during inflammatory conditions in response to cytokines such as tumor necrosis factor α, interleukin 1, and interferon γ. Regulation of this isoform is under the control of the transcription factor nuclear factor κB.

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The importance of nitric oxide spurred the development of assays to monitor its production *(1)*. The site of nitric oxide production was initially determined by the detection of NOS isoforms. The activity of NOS was first determined by use of enzyme histochemical assays and after the development of antibodies by immunohistochemical methods, although the development and use of antibodies proved to be challenging *(2)*.

Nitric oxide is a short-lived radical and readily reacts with small scavenger molecules (e.g., uric acid, glutathione) and macromolecules (proteins, DNA) or is oxidized into nitrite and nitrate. Therefore, the detection of the radical itself proved very difficult, and only labor-intensive, sophisticated spin-trapping methods measured actual production of the nitric oxide radical. Assays for the stable reaction products of nitric oxide were also developed. Nitric oxide radical readily reacts with reactive oxygen species such as superoxide anions to yield peroxynitrite, an extremely reactive and toxic compound that is able to nitrosylate tyrosine residues in proteins, leading to the formation of modified proteins with changed or inhibited function. This nitrotyrosine modification can be detected using specific antibodies.

We were interested in nitric oxide's role in the liver during systemic inflammatory conditions (endotoxemia, a model of sepsis) and in acute rejection after liver transplantation *(3, 4)*. More specifically, we were interested in the effects of NOS inhibitors on liver function in these conditions *(3)*. To assess the effect of NOS inhibitors, we needed a reliable method to measure nitric oxide metabolites. We opted for an assay based on the Griess reagent, which is specific for nitrite, and we validated this assay for use with plasma samples. Our main findings were: (*a*) nitric oxide was predominantly and rapidly oxidized to nitrate rather than nitrite, and therefore it was useless to apply the Griess assay without prior reduction of nitrate to nitrite using nitrate reductase; (*b*) plasma and serum samples needed to be deproteinized to eliminate artifacts; and (*c*) the assay proved to be reliable and reproducible, and the recovery of nitrate and nitrite from plasma by use of our Griess-based assay was approximately 90%. We subsequently modified this assay for other body fluids such as urine and sputum *(5)*. In addition to the Griess assay, HPLC methods were developed to measure nitrite and nitrate. The disadvantage of methods measuring

stable end products is that actual nitric oxide production from NOS cannot be determined. Methods have also been developed that use stable isotopes to measure nitrite and nitrate derived from nitric oxide. These methods are, however, rather labor-intensive and complicated.

Although the interest in nitric oxide may have somewhat diminished over the last decade, methods to measure nitric oxide (metabolites) are still used extensively.

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