

# Nitric oxide and its role in health and diabetes.



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## Part 3. NO (NITRIC OXIDE) METABOLISM IN DIABETIC PATIENTS.

To understand NO metabolism in diabetic patients we must first discuss the normal process of NO formation. NO is a gas that is also a short-lived, unstable free radical and, within seconds of production, must become stabilized. To do so, it reacts with one or more elements or biologic compounds, as described below.

First, NO may diffuse into smooth muscle cells and bind to an enzyme called guanylate cyclase (GC). As discussed in more detail in the next article in this series, this binding to GC initiates the process of vasodilation.

Secondly, NO can interact with oxygen to form the stable nitrates and nitrites that are measurable in serum, urine, or saliva. Clinically, nitrates and nitrites reflect the status of a particular patient in producing NO at the time the measurement is made. Higher concentrations of nitrate and nitrite are suggestive that high amounts of NO have recently been produced.

Lastly, NO may bind to sulfur (S) elements that are found as a part of certain amino acids, such as cysteine, and in other biologic compounds, such as glutathione, a well-recognized anti-oxidant. This binding to S molecules results in the formation of nitrosothiols. (A nitrosothiol is another name for a compound in which NO is attached to sulfur). Later, the NO can be released from the nitrosothiols to cause biologic responses such as smooth muscle cell relaxation and vasodilation.

Hemoglobin is a protein within RBC and is made up of two alpha chains and two beta chains. Although hemoglobin is well known for its ability to carry oxygen to tissues, a less well-appreciated fact is that on the beta chain are cysteine amino acids (which contain S) that bind NO as nitrosothiols. Thus hemoglobin carries both NO, which may be subsequently released, and oxygen.

Both Type I and Type II diabetic patients have a reduced ability to generate NO from L-arginine, reflected in part by direct measurements of plasma nitrate and nitrite levels. Several factors influence nitric oxide production and metabolism. Because NO is derived from the amino acid L-Arginine, one of the amino acids that make up proteins, it is clear that adequate protein intake is essential for NO production. However, simply adding more L-arginine to the diet of diabetic patients may not solve the problem of low NO production.

First, as part of normal metabolism of L-arginine small amounts of a natural inhibitor of NOS are formed. These inhibitors do not accumulate in the blood because they are rapidly eliminated in the urine provided kidney function is normal. The major inhibitor is named asymmetrical dimethyl arginine (ADMA). ADMA does, however, accumulate as kidney function declines and many diabetic patients lose kidney function as part of the disease process. Therefore, increasing dietary L-arginine, in an attempt to increase NO production, may be counterproductive in diabetic patients with decreased kidney function. Reduced kidney function is a part of aging (more than 24% of all Americans over 65 have Type 2 diabetes) and kidney dysfunction, which is accelerated by diabetes, may prevent the elimination of the major NOS inhibitor, ADMA. In this case, the production of NO would be low because NOS activity was inhibited by ADMA.

From our last article, you will recall that NO is produced from L-arginine due to the enzymatic activity of nitric oxide synthase (NOS). NOS is a pH (acid/base measurement) dependent enzyme; it is active at slightly alkaline (basic) conditions but is suppressed by acidotic conditions. In diabetes, glycolysis and ketoacidosis force pH toward acid conditions and this may account, in part, for the reduced production of NO since a slightly basic pH is ideal for NOS enzymatic activity.

Oxygen is a cofactor for the activity of NOS and therefore adequate oxygen is necessary for NO production. In the absence of sufficient oxygen there is less NO produced because the enzyme NOS will not function as well as normal. Circulation (in other words blood flow that brings oxygen to a particular site) is notoriously impaired in diabetic patients. One can appreciate the magnitude of the reduced blood flow, and the concomitant reduction in oxygen delivery, with non-invasive modalities such as scanning laser Doppler, TcPO<sub>2</sub>, or ABI measurements.

Plasma nitrate and nitrite concentrations are often lower in both Type I and Type II diabetic patients than in normal subjects thus indicating lower levels of NO production, irrespective of whether kidney function is below normal. JV Boykin, Jr., M.D. recently made an interesting observation. He noted that diabetic patients who didn't heal with growth factor therapy (to be discussed in a later article in this series) had very low levels of nitrates and nitrites in their urine, whereas those that did heal had higher, near normal concentrations of urinary nitrates and nitrites. The interpretation was that failure to heal a diabetic ulcer might be related to low rates of NO production. It is not clear yet whether, in diabetic patients, low L-arginine intake, acidosis, low oxygen, or accumulation of ADMA, or all of these are responsible for the **decreased production of NO**, reflected by low urinary nitrate and nitrites. Most likely, all these events are occurring simultaneously.

In diabetes, glucose levels are elevated. Some of this glucose becomes incorporated into hemoglobin and is measured as glycosylated hemoglobin (Hgb) or HgbA1C. Glycosylated hemoglobin binds NO in the form of nitrosothiols very tightly so that **any NO that is formed cannot be easily released** from RBC to help maintain blood flow through smooth muscle cell relaxation.

To summarize, it is clear in diabetic patients that acidosis, low oxygen, and/or accumulation of ADMA are responsible for the decreased production of NO. Tighter binding to glycosylated hemoglobin may also limit release of NO to the plasma and smooth muscle cells. Most likely, all these events are occurring simultaneously and they account for the low plasma and urine levels of nitrates and nitrites in diabetic

patients. Reduced production and higher than normal binding, may be partly responsible for the poor circulation in diabetic patients and would be one of the reasons for their high propensity to develop an ulcer. In the next article, we will discuss the mechanism by which NO causes vasodilation.

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