

Urinary sulfate, asymmetric dimethylarginine, and 8-hydroxy-2'-deoxyguanosine: markers of metabolic imbalances and nutritional need

J. Alexander Bralley, Ph.D., CCN

Denise Autry, RN, CCN

ABSTRACT

Compounds found in the urine represent the end products of metabolism and are the expression of the efficiencies or inefficiencies of this process. Nutritional status has a direct effect on this metabolic efficiency. Careful assessment of certain urinary compounds can yield valuable information on the nutrient needs of the individual. Urinary sulfate, asymmetric dimethylarginine (ADMA), and 8-hydroxy-2'-deoxyguanosine (8-OHdG) are critical markers of metabolic function, reflecting the status of multiple systems in the human body due to the broad-based nature of their biological influence. Sulfate is utilized in the detoxification and conjugation of both endogenous and exogenous compounds. ADMA is a potent inhibitor of nitric oxide synthase, consequently affecting the production of nitric oxide and its associated systemic functions. Oxidative damage to DNA is reflected in the urinary excretion of the oxidized damage adduct, 8-OHdG. Each one of these compounds can be influenced by nutritional status. Measurement of these end products in urine provides an insight into specific nutrient needs of the individual and identifies prospective areas of support.

Introduction:

Nutritional status has a profound influence on health. From frank nutrient deficiency diseases to the development of many chronic degenerative illnesses due to subclinical insufficiency, nutrition is a critical component of long term health status. Defining the appropriate nutrient needs for the individual is a challenging task. The principle of biochemical individuality yields a wide range of nutrient requirements for optimal function in the population. What may be appropriate for one individual may not necessarily be what is needed for another.

As just one example, research has noted that mutant enzymes with poor binding affinity account for more than 50 known genetic conditions. These conditions, however, can often be stabilized with appropriate nutrient intakes. In individuals with these defective enzymes, the administration of high doses of the vitamin component of the corresponding coenzyme partially restores enzymatic activity. [1]

With these kinds of variations, how does one determine optimal nutrient needs for the individual? Measurement of nutrient levels in various compartments of the body has been one approach to nutritional assessment. Blood, urine, or tissue levels of vitamins and minerals can give the level of a particular nutrient present. However, comprehensive testing of all important nutrients is expensive and requires large amounts of specimen, which may not be easily attainable.

Many compounds in the urine are the end products of metabolism. These urinary compounds reflect metabolic processes, many of which can be modulated by nutritional factors. [2] Urinary metabolites often indicate the "functional level" of need for a specific nutrient, not merely an absolute level, which can be misleading. For example, homocysteine is a molecule that requires specific vitamins for metabolism. It becomes elevated in blood when a functional deficiency of B12, folate, or B6 disallows further metabolism. Research has shown that when blood levels for these vitamins are measured, they may be within normal reference limits *even though blood homocysteine levels are high*. [3] These "normal" results would lead to an inaccurate evaluation of the need for compensatory supplementation in the high-risk homocysteinemic population. Functional need for proper nutrient intake is, therefore, better indicated by the measurement of intermediate compounds which require them for their metabolism. High levels of these intermediate compounds in the urine would more accurately indicate the functional need for critical metabolic nutrient co-factors.

Urinary sulfate, ADMA, and 8-OHdG yield significant metabolic information and are easily collected and measured. These analytes provide useful data on the functional nutrient needs of an individual. They are not direct measures of nutrients themselves; but they can be significantly affected by specific nutrient status and, therefore, provide positive information on individual nutrient insufficiencies. While not providing a comprehensive assessment of all nutrients required by the body, they do provide a good reflection of functional nutrient needs in several critical areas of metabolic function. When this information is combined with age and gender, plus a review of specific lifestyle and dietary habits, a very useful nutrient supplement profile can be developed that addresses the unique nutrient needs of the individual.

The following is a brief review of these three compounds with a rationale for their use as indicators of specific nutrient need. A selected annotated bibliography is provided to illustrate and elaborate upon major points in the text.

Sulfate:

Inorganic sulfate is used in the body for detoxification of normal metabolic by-products, hormones, drugs, and other foreign compounds circulating in the blood. Sulfate is conjugated with these compounds as part of Phase II detoxification reactions, which occur primarily in the liver. This step renders the molecules more water soluble so that they can circulate in the blood and be excreted from the body via the kidneys into the urine. Sulfate is primarily derived from the sulfur-containing amino acids in dietary protein, methionine and cysteine. Other Phase II conjugation compounds include glutathione, glycine, and glucuronic acid. Glutathione is a tripeptide containing cysteine and is, therefore, also cysteine/methionine dependent. Sulfate is converted in the liver to the active co-substrate for conjugation, adenosine 3'-phosphate 5'-phosphosulfate (PAPS).

Research indicates that sulfate tissue levels can be influenced by certain dietary regimens. Protein calorie malnutrition, fasting, and low-protein diets can deplete inorganic sulfate stores and glutathione levels due to the decreased availability of methionine and cysteine. [4] In addition, excessive exposure to foreign chemicals can deplete sulfate stores and lead to chronic illnesses. [5, 6] Sulfate levels are strictly determined by dietary protein intake and its subsequent digestion and assimilation of the amino acid components, cysteine and methionine. This process may become compromised in certain individuals, especially those on weight loss, low protein, or other low-calorie diets. Malabsorption/maldigestion of protein and consequent amino-acid imbalance is a relatively common phenomenon in the US, particularly with the overuse and prevalence of antacids and acid-blocking drugs.

Low urinary sulfate levels can indicate depleted body stores and a functional need for supplementation of sulfur-containing amino acids as well as support for other detoxification processes. Urinary sulfate directly reflects the circulating levels and availability of this important detoxification compound. [5] Evidence suggests that as the sulfation pathway is depleted, other Phase II conjugations can take place to compensate. [7, 8] These concepts have been well documented in the literature on acetaminophen detoxification. [9-17] Sulfate and glutathione depletion significantly increases the toxicity of acetaminophen. This toxicity can be reversed with adequate supplementation of the precursor amino acids such as cysteine or its more easily-absorbed form, N-acetylcysteine. Glycine or glutathione supplementation may also be useful, as this would support alternative detox pathways which may also be under stress. As sulfate and glutathione levels are repleted in the body, urinary levels of sulfate are also normalized.

ADMA:

Nitric oxide (NO) is a biologically active molecule which has generated much enthusiasm in scientific circles. It was deemed "Molecule of the Year" in 1992 by Science magazine, and the Nobel Prize in Medicine and Physiology was awarded to its major researchers in 1998. Nitric oxide regulates many bodily processes in response to a variety of stimuli. NO is involved in blood flow regulation, maintenance of vascular tone, and control of platelet aggregation. It is a rapid-response neurotransmitter in the central nervous system and influences cerebral blood flow and synaptic plasticity. [18] In response to infection or inflammatory processes, nitric oxide is produced in large amounts in macrophages, a type of white blood cell involved in immune system defense. Macrophages are important factors in destroying cancers and infectious organisms. Nitric oxide can directly kill parasites and bacteria, as well as fungal organisms. [19] Microbes are kept in check by proper production of NO by immune system cells in many parts of the body. [20] In the gastrointestinal tract, NO regulates the contractions of smooth muscle tissue and promotes normal peristalsis. [18] Nitric oxide is a potent vasodilator and is thought to be a fundamental regulator of vascular tone by its ability to stimulate arterial endothelial cells to relax the smooth muscle of the arteries. [21, 22] This effect is seen in the use of nitroglycerin for angina pain. Nitroglycerin is rapidly converted to NO which relaxes the

coronary arteries, increasing blood flow and reducing chest pain. Suboptimal production of NO in the cardiovascular system has been linked to the development of hypertension.

Conversely, overproduction of NO can produce negative effects. Nitric oxide and superoxide are produced concurrently in microbial-induced immune activation and other inflammatory processes. These molecules react with one another to produce the very reactive pro-oxidant, peroxynitrite. The microbe-destroying mechanism of peroxynitrite production is cytotoxic to normal tissues, especially in conditions of chronic overproduction of NO. This potent free-radical oxidant can damage cellular membranes as well as other cellular components. [23] Peroxynitrite also reacts with sulfhydryl groups thereby depleting such compounds as glutathione, [24] lipoic acid, cysteine, and other sulfhydryl-containing enzymes and proteins. Peroxynitrite also depletes coenzyme Q10 and increases lipid peroxide formation. Nitric oxide will bind to vitamin B12, potentially leading to B12 deficiency, which can have serious ramifications for nervous system myelination.[25-27] Elevated NO concentrations are found in numerous gastrointestinal pathologies, including peptic ulcer, chronic gastritis, gastric cancer, and inflammatory bowel disease. [18] GI luminal NO production has been shown to be 100 times higher in persons with ulcerative colitis.[28] Sustained levels of NO production result in direct tissue toxicity and contribute to the vascular collapse associated with septic shock [29], whereas chronic expression of NO is associated with various carcinomas and inflammatory conditions including juvenile diabetes [30], multiple sclerosis [31], arthritis [32, 33], and ulcerative colitis. [28] Excessive NO can lead to oxidant stress, which can damage coronary arteries leading to atherogenesis. [34]

Therefore, it is clear that NO is a potent regulatory molecule. Either over- or under-production of NO can have significant effects on body function. Consequently, its proper regulation has been the subject of much study.

The regulation of NO is dependent upon a family of enzymes that convert the amino acid L-arginine to L-citrulline and NO. Arginine is converted to NO by the enzyme nitric oxide synthase (NOS), which is present in three forms in the body: neuronal (nNOS or type I), inducible (iNOS or type II), and endothelial (eNOS or type III). Two of the forms (nNOS and eNOS) are considered constitutive and iNOS is — hence the name — inducible. [35, 36] The constitutive forms are present in a fixed amount to provide a constant level of NO to a system. The inducible form is generated, via standard DNA/RNA protein synthetic cellular machinery, in response to a specific stimulus. This allows the body to create additional amounts of NO in response to an increased demand, usually signaled by the presence of an endotoxin and/or proinflammatory cytokines.

As part of this regulatory mechanism, NOS activity can be actively limited by the endogenous inhibitor, asymmetric dimethylarginine, also formed from arginine. Nitrogen atoms of arginine residues can be covalently mono- or dimethylated by the action of protein arginine methyltransferases (PRMT), a recently discovered gene family [37]. Under the influence of PRMTs, select target proteins are methylated in various combinations. Di-methylation produces asymmetric dimethylarginine (ADMA).

ADMA production must be carefully controlled in the body since ADMA easily modulates the body's production of nitric oxide. ADMA has been shown to be an endogenous competitive inhibitor of all isoforms of NOS. [38] If ADMA levels become too high, significant effects can occur. In humans, elevated ADMA can cause increased vascular resistance, decreased blood flow in the brain, increased sodium retention, and decreased cardiac output, among many other effects. Circulating ADMA levels have been assessed in a variety of systemic cardiovascular diseases, and are significantly increased in conditions associated with hypoxia, renal failure, pulmonary hypertension, heart failure, and hypercholesterolemia, [39, 40] insulin resistance [41], diabetes mellitus [42], hyperhomocysteinemia [43], hypertension [44], and endothelial dysfunction [22,45]. Gene researchers have identified 979 genes that change expression when exposed to high levels of ADMA. [46]

Measuring ADMA levels specifies those individuals more likely to benefit from targeted nutrient support for the biological consequences of either inhibited nitric oxide synthesis (indicated by elevated levels of ADMA) or the potential sequelae of excessive NO production (as a result of decreased ADMA). Since excess NO is related to inflammatory processes in the body and inadequate nitric oxide is associated with susceptibility to infection and vascular issues, the measurement of ADMA provides a guideline for the specific aspects of nutritional support required by the individual.

Structurally NOS enzymes are very similar to the cytochrome P-450 enzymes used in the liver for detoxication. P-450 enzymes can be modulated by nutritional factors.[47] Similarly, various nutritional factors have been shown to influence NOS production [48-55] and the effects of NO. [56-60]

In cases of excessive NO production, antioxidant supplementation with vitamin C, E, and glutathione has been shown to reduce oxidant stress imposed by peroxynitrite production. [57] Addition of glycine as a glutathione precursor can increase glutathione synthesis. [61] Supplementation of B12 could also help reduce NO levels in situations of overproduction of NO. [26, 27] Omega-3 fatty acids can suppress the activation of inflammatory prostaglandins associated with excess NO. [56] These nutrients, along with the referenced antioxidants, may be useful in controlling the consequences of excess NO output in tissues. [58]

For those exhibiting increased ADMA and consequent depressed NO production, other nutrients are indicated. Garlic's beneficial effects on cardiovascular function are thought to be caused in part by its ability to stimulate NO production by activation of NOS. [53, 54, 62, 63] Supplemental arginine has been used to increase NO output and reduce blood pressure and improve circulation. [49, 64-67] In hypercholesterolemic experimental animals, arginine supplementation to raise NO levels has been shown to lower cholesterol and reverse atherosclerotic plaquing of coronary arteries better than a cholesterol lowering drug.[68] Endothelial function in the coronary arteries is impaired in hypercholesterolemic individuals, which leads to the formation of atherosclerotic lesions. Additional experimental data suggest this impaired function can be corrected by supplementation with arginine, if the individual is low in arginine and/or NO synthesis.[69-71]

8-hydroxy-2'-deoxyguanosine (8-OHdG):

Reactive oxygen species (ROS) are ions formed by the incomplete one-electron reduction of oxygen. These reduction/oxidation (redox) reactions are vital to life. ROS are known mediators of intracellular signaling and gene expression. The immune system utilizes ROS intermediates, such as peroxynitrite, singlet oxygen, and the hydroxyl radical, in the microbicidal activity of phagocytes. However, excessive production of ROS may lead to oxidative stress and damage to nucleic acids, proteins, and lipids, resulting in loss of cellular function and cell death. [72]

Due to the vulnerability of nuclear and mitochondrial DNA to oxidative damage, biological systems have developed effective repair systems. During DNA repair, specific repair adducts are released into the circulation for excretion through the kidneys. After almost 25 years of genetic study, the most frequently detected and studied DNA repair product is 8-hydroxy-2'-deoxyguanosine (8-OHdG), an oxidized nucleoside of DNA guanine. Out of all the identified sugar- and base-derived DNA lesions, the biomarkers of oxidant-stress exposure and levels of 8-OHdG are the most closely validated. [73]

The measurement of 8-OHdG is considered a biomarker of cellular oxidative stress throughout the body and studies increasingly indicate that elevated levels may be a risk factor for cancer, atherosclerosis, diabetes, and other health challenges. For example, an elevated level of urinary 8-OHdG has been identified as a factor of initiation and promotion of carcinogenesis.[74, 75] Elevated 8-OHdG is found in cases of chronic inflammation due to the continuous formation and accumulation of ROS in DNA, leading to various human pre-neoplastic lesions.[76] Increased levels of 8-OHdG are found in human atherosclerotic plaques. Elevated urinary 8-OHdG was also identified in poorly-controlled diabetic patients, with the level of urinary 8-OHdG in diabetes correlating with the severity of diabetic nephropathy and retinopathy. [77] Researchers have identified increased 8-OHdG levels as a potential factor in the development of comorbid medical conditions in depressed individuals. [78] A common cause of male infertility is oxidative stress-induced sperm damage, through the initiation of apoptosis or the direct oxidation of the DNA by ROS. [79] Age-associated decline in mitochondrial function due to mitochondrial DNA damage is indicated by the finding of increased DOG with age in laboratory animals. [80]

Environmental conditions can promote the formation of ROS, as reflected by increased urinary 8-OHdG. Bus drivers from Copenhagen showed increased levels when compared with drivers from rural/suburban Copenhagen. [81] Exposure to UV light (sunlight) and benzo[a]pyrene (an ubiquitous compound found in automobile exhaust, in all smoke resulting from the combustion of organic material, and in charbroiled food) can synergistically enhance the formation of 8-OHdG in living cells.[82] Based on increased 8-OHdG levels, a young and healthy population of boilermakers experienced an increased risk of developing oxidative DNA injury after exposure to high levels of metal-containing particulate matter. [83]

The measurement of 8-OHdG can aid in identifying those individuals who might benefit from nutritional support for increased ROS production. A variety of cellular antioxidants have been identified that specifically eliminate ROS. These include superoxide dismutases, catalases, lipoic acid, glutathione (a tripeptide composed of glycine, glutamic acid, and cysteine), alpha-tocopherol, and vitamins A and C.[84] Glutathione and ascorbate can protect human lymphocytes against oxidative DNA damage. It is logical to assume, then, that inadequate intakes of vitamins and minerals can lead to DNA damage, mitochondrial decay, and other pathologies [85] Studies show that antioxidant levels are negatively correlated with the levels of oxidative lymphocyte DNA damage. [86] Inadequate intake of folate, B12, or B6 leads to uracil incorporation into DNA and chromosome breaks, which mimics damage done to DNA by radiation.[87, 88]. In human cell cultures, inadequate zinc causes release of oxidants, producing oxidative damage to DNA and inactivation of p53 and other zinc enzymes involved in DNA damage repair [89, 90]. Exercise is a known inducer of ROS and studies in athletes show that Vitamin E supplementation may reduce DNA oxidation induced by training. [91] Biotin deficiency in human cells in culture leads to oxidant release, DNA damage, accelerated mitochondrial decay, and premature senescence [92]. Magnesium deficiency in human cells in culture causes mitochondrial DNA protein crosslinks, accelerated telomere shortening, and premature senescence [93]. Oral antioxidant therapy in infertile men has been found to improve sperm DNA integrity, while reducing apoptosis and seminal ROS production.[79] Even among populations that might be considered normal, increasing the intake of antioxidant vitamins with a nutrient-rich supplement reduces 8-OHdG by 22% compared with un-supplemented adults.[94]

Conclusion:

Measurement of urinary sulfate, asymmetric dimethylarginine, and 8-hydroxy-2'-deoxyguanosine provide useful information on functional need for nutrient supplementation. Sulfate levels reflect adequacy of sulfur amino acid and glutathione stores in the body, critical nutrients for detoxification, antioxidant protection, and structural maintenance. Low levels would provide justification for supplementation of cysteine, taurine, B6, B5 and/or glycine. The oxidant stress level of the individual and the need for adequate antioxidant protection is reflected in levels of ADMA and 8-OHdG. Too little or too much NO production, as indicated by ADMA levels, can adversely affect healthy function of multiple systems in the body. Low NO can be raised with arginine supplementation. The negative effects of oxidant stress due to excessive NO can be mitigated by specific nutrient support, including flavonoids, B-complex, and antioxidant vitamins and minerals. B12 can bind with NO and may be a useful adjunct in NOS up-regulated individuals where overproduction of NO is causing oxidant stress. Specific antioxidant nutrients are useful in mitigating the effects of DNA damage by ROS and can be valuable in facilitating the DNA repair process.

These urinary compounds constitute useful biomarkers of nutritional need that provide a logical scientific basis for determining individual nutritional requirements.

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