Physiological effects of nitrate, nitrite and nitric oxide: An Overview on recent insights

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Summary: Inorganic nitrate (NO_3^-) and nitrite (NO_2^-) are part of the nitrogen cycle in nature. To toxicologists and the general public these anions are generally known as undesired residues in the food chain with potentially carcinogenic effects (1). Among NO biologists, these inorganic anions have merely been viewed as inert oxidative end products of endogenous nitric oxide (NO) metabolism (2). However, more recent studies surprisingly show that nitrate and nitrite can be metabolized in vivo to form nitric oxide (NO) and other bioactive nitrogen oxides (3-5). This represents an important alternative source of NO especially during hypoxia when the oxygen-dependent L-arginine-NO pathway can be dysfunctional (6). These novel lines of research suggest important biological functions of the nitrate-nitrite-NO pathway with profound implications in relation to the diet and cardiovascular homeostasis. Nitrate in dietary doses reduces blood pressure in healthy volunteers as well as in hypertensives (7-9). In addition nitrate increases the efficiency of mitochondrial respiration (10), inhibits platelet aggregation (11) and improves peripheral blood flow in peripheral artery disease (12). Moreover, an increasing number of studies suggest a therapeutic potential for nitrate and nitrite in diseases such as myocardial infarction, stroke, hypertension, renal failure and gastric ulcers (13, 14). A theory is emerging suggesting nitrate as an active component in vegetables contributing to the beneficial health effects of this food group (15), including protection against cardiovascular disease and type-2 diabetes. Toxicologists and authorities advocating merely negative health effects of nitrate, must now seriously take these opposing new data into consideration when attempting to judge the overall impact of dietary nitrate on human health.

The Nitrate-Nitrite-NO Pathway: Mammalian NOS-independent NO generation from nitrate was first demonstrated in 1994 by two independent groups (16, 17). Nitrate from endogenous (NOS) and exogenous (diet) sources circulates in blood and is actively taken up by the salivary glands and excreted in saliva. In the oral cavity nitrate is reduced to nitrite by commensal bacteria and the nitrite formed is continuously swallowed into the acidic lumen of the stomach (18, 19). Large amounts of NO and other reactive nitrogen oxides are immediately formed in the stomach upon acidification and these species have profound effects on the gastric mucosa, including stimulation of mucosal blood flow, mucus generation and inhibition of pathogen growth (13). Remaining nitrite is absorbed systemically and numerous non-enzymatic and enzymatic pathways help to further reduce the nitrite to NO in blood and tissues (5). Oral bacteria are essential for the bioactivation of dietary nitrate and removal of these bacteria by an antiseptic mouthwash effectively blocks nitrite formation and the biological effects of nitrate (20, 21).

Cardiovascular and metabolic effects of dietary nitrate: In 2006 Larsen and colleagues showed that blood pressure is reduced in healthy volunteers after ingestion of nitrate at doses easily achievable with a diet rich in vegetables (7). Webb and colleagues then noted substantial BP-lowering effects of a natural nitrate source (beetroot juice) (8) and these studies have since been confirmed by numerous groups (22). NO formation from nitrate also has other effects on the cardiovascular system including increases in peripheral blood flow, inhibition of platelet function and improvement of vascular function (measured as increases in flow-mediated dilation, FMD).

Interestingly, dietary nitrate also has metabolic effects. Larsen et al. demonstrated that the amount of oxygen consumed during physical exercise is decreased by nitrate (23), a finding that has been reproduced in numerous studies (24-26). The mechanism of this remarkable effect seems to be a nitrate-induced increase in mitochondrial efficiency so that more ATP is generated for every oxygen molecule consumed thereby making muscular work more efficient (10). Studies now also show that athletic performance is increased by these mechanisms (27). Although not yet studied in detail, this could be of importance not only for competitive athlese but also for certain groups of patients characterized by oxygen deficiency, including those with peripheral artery disease (PAD), mitochondrial diseases and chronic obstructive lung disease. Animal studies have also shown other interesting metabolic effects of nitrate including reversal of the metabolic syndrome-like state seen in mice lacking endothelial NO synthase (28), and stimulation of insulin release from pancreatic islets (29).

Summary and future perspectives

During the last decade we have witnessed a veritable paradigm shift in how we are viewing dietary nitrate in relation to human health. For > 50 years this inorganic anion, abundant in vegetables, has been considered to be solely a toxic unwanted residue in our food chain. Then with the discovery of the L-arginine/NO pathway, scientists began to view nitrate merely as an inert end product of NO metabolism. Now the picture is beginning to change again with the discoveries of potential beneficial effects of nitrate, especially in relation to cardiovascular function. These effects of depend on nitrate bioactivation by oral bacteria which generate the more reactive nitrite anion. Nitrite is partly absorbed systemically and can be further metabolised to NO and other bioactive nitrogen oxides in blood and tissues. Nitrate still has a terrible reputation world wide which is mainly based on the proposed relationship with nitrosamine formation and development of cancer. Importantly however, such effects of nitrate have not been substantiated despite immense efforts over the past five decades. With the emergence of new data showing possible positive effects of nitrate on the cardiovascular system, we need to balance the discussion and also consider such effects when judging the overall impact of nitrate on human health. It is of outmost importance that toxicologists, biochemists, physiologists and other researchers begin to communicate and cooperate with each other to finally settle the role of dietary nitrate in health and disease. Regulatory authorities should be involved in these discussions and ultimately the goal would be to establish solid recommendations regarding allowable levels of nitrate in food and drinking water, acceptable (or recommended) daily intake, and general advice on how nitrate affects human health.

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