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Dietary Nitrite: from menace to marvel

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ABSTRACT:

The health benefits of nitrite are now indisputable when administered in a clinical setting for specific diseases. Currently, most published reports identify the production of nitric oxide (NO) as the mechanism of action for nitrite. Basic science, in addition to clinical studies, demonstrate that nitrite and/or nitrate cannot restore NO homeostasis as an endothelium independent source of NO that may be a redundant system for endogenous NO production. Nitrate must first be reduced to nitrite by oral commensal bacteria; nitrite can then be further reduced to NO along the physiological oxygen gradient. But despite decades of rigorous research on sodium nitrate's safety and efficacy as a curing agent, sodium nitrite is still regarded by many as a toxic undesirable food additive. However, research within the biomedical science community has revealed enormous therapeutic benefits of nitrite which are being developed as novel therapies for conditions associated with nitric oxide insufficiency. Thus, this review will highlight the fundamental biochemistry of nitrite in human physiology and provide evidence that nitrite be considered an essential nutrient. Foods or diets enriched with nitrite can have profound positive health benefits.

Keywords: nitrite, nitrate, nitric oxide, curing, nutrition, epidemiology, cardiovascular, cancer, diet, nitrosamines, antioxidants

INTRODUCTION

Imagine if a single dietary component was proven to be able to protect one from injury from heart attack [1], reverse hypertension [2, 3], enhance insulin signaling and glucose uptake while reversing features of metabolic syndrome [4, 5], inhibit microvascular inflammation, reverse endothelial dysfunction and reduce levels of C-reactive protein [6], kill food borne pathogens [7, 8], enhance gastric mucosal blood flow [9], prevent ulcerative colitis [10] and inhibit cancer cell

growth and proliferation [11, 12]. These are just a few of the physiological functions of dietary nitrite which have been revealed over the past decade [13]. However, negative press over the previous 50 years has dampened the enthusiasm for these revolutionary discoveries and are consequently met with some resistance. Therefore, a change in paradigm is required. The nitrite molecule, which was once considered a potentially toxic food additive that should be avoided, is now considered to be an essential nutrient by many individuals in science. This review will highlight the health benefits of dietary nitrite, while also discussing mechanisms to prevent nitrosative chemistry and subsequent nitrosamine formation.

Nitric Oxide Biochemistry and Physiology: Before we review the underlying biochemistry of nitrite in human physiology, it is first necessary to describe the fundamental roles and production pathways for NO and its implications in health and disease in order to better appreciate the new found and beneficial role of nitrite. The mammalian biosynthesis of NO discovered in the 1980's for its roles in the immune [14, 15], cardiovascular [16-18], and nervous [19] systems established a new, startling paradigm in the history of cellular signaling mechanisms. Prior to this discovery, NO was widely considered and recognized as a toxic molecule. In the past, NO was identified as a common air pollutant, a constituent of cigarette smoke, and a toxic gas, which appears in the exhaust of motor cars and jet airplanes, causes acid rain, and destroys the ozone layer. Consequently, it was essentially inconceivable that cells would intentionally produce this toxic gas as a part of normal physiology. A similar phenomenon is now occurring regarding nitrite. Although nitrate was once considered to be a harmful and toxic food additive implicated in cancer, it is now acknowledged to be a beneficial molecule with medicinal properties that positively affect many organ systems. NO is now recognized to be one of the most important signaling molecules in the body, being involved in virtually every organ system in which it is responsible for modulating an astonishing variety of effects. The primary targets for NO are thiols [20] or iron/copper-containing proteins [16]. NO can bind to soluble gualylyl cyclase (sCG) and cause an increase in second messenger cGMP [16] and mediate a number of physiological functions. This pathway was considered to be the basis of NO based signaling, until it was discovered that NO elicited a number of physiological and biological effects that were not dependent upon cGMP. It is now recognized that NO can react directly with thiyl radicals to form nitrosothiols or other reaction products of NO i.e. nitrite, N_2O_3 or N_2O_4 that can post-translationally modify thiols to affect protein structure and function [21]. NO has been shown to affect and be involved with practically every organ system in the body [22]. Therefore, one can imagine how a host of diseases, conditions, and multi-systemic symptoms may be caused or affected by the body's dysregulation of NO production/signaling. Maintaining NO homeostasis is critical for optimal health and disease prevention. Nitrite may be fundamental in maintaining NO homeostasis through reduction to NO and through maintenance of nitrosothiols [23, 24].

The consequences of NO insufficiency are broad and profound. The continuous generation of NO is essential for the integrity of the cardiovascular system; additionally, the decreased production and/or bioavailability of NO is central to the development of many disorders [22]. Aging is considered the single largest risk factor related to cardiovascular related diseases and deaths. Cardio-protection decreases with increasing age and is attributed to a decline in NO. The

lack of NO production can lead to hypertension, atherosclerosis, peripheral artery disease, heart failure, and thrombosis leading to heart attack and stroke, the leading cause of death for all Americans. Remarkably, all of these conditions have been shown to be positively affected by dietary nitrite interventions [13, 25, 26].

Nitrite in Human Physiology: Although the L-arginine-NO pathway was the first to be discovered, it does not necessarily mean it is the primary pathway for the endogenous production of NO. In fact, nitrite may be central in the maintenance of NO homeostasis. The activation and metabolism of nitrite in human physiology requires hemoproteins [27], similar to the known chemistry in meat curing. This is the historical basis for the addition of sodium nitrite into ready-to-eat meat and cured meat products. Research performed over the past decade realized that nitrite is physiologically recycled in blood and tissues to form NO and other bioactive nitrogen oxides [23, 28-30]. Nitrite is an oxidative breakdown product of NO that has been shown to serve as an acute marker of NO flux/formation [31]. Nitrite is in steady state equilibrium with S-nitrosothiols [23, 32] and has been shown to activate sGC and increase cGMP levels in tissues [23], activities very similar to NO. In the early 1980's, it was demonstrated that, in addition to dietary exposure, nitrite is also generated endogenously [33]. Shortly thereafter, the entire L-arginine-nitric oxide synthase (NOS)-system was discovered and found to be the major endogenous source of nitrite, since NO is rapidly oxidized to nitrite [15]. Once nitrite is produced and circulated, it is taken up by peripheral tissues and can be stored in cells. The 1-electron reduction of nitrite can occur by ferrous heme proteins (or any redox active metal) through the following reaction:

$$\mathbf{NO_2^- + Fe^{(II)} + H^+ \quad \leftrightarrow \quad NO + Fe^{[III]} + OH^-}$$

This is the same biologically active NO which is produced by NOS, with nitrite rather than L-arginine as the precursor. However, nitrite reduction along the physiological oxygen gradient is a relatively inefficient process [24]. It is now clear that nitrite is recycled *in vivo* and again forms bioactive nitrogen oxides, including NO [35-40]. Thus, instead of simply wasting the products of NO oxidation, mammals store and actively recycle it. Human nitrite reduction to NO was first described in the stomach, where salivary nitrite forms NO non-enzymatically via acid-catalyzed reduction [29, 30]. Shortly after this observation, Zweier described NOS-independent nitrite reduction in the ischemic and acidic heart [41]. In the last 15 years, it has become evident that blood and tissue nitrite is reduced under physiological conditions to form NO and nitrosothiols and modulate blood flow [42, 43].

Much of the recent focus on nitrite physiology is due to its ability to be reduced to NO during ischemic or hypoxic events [44-46]. Nitrite reduction in mammalian tissues has been linked to the mitochondrial electron transport system [47, 48], protonation [45], deoxyhemoglobin [43], and xanthine oxidase [49, 50]. Nitrite can also transiently form nitrosothiols (RSNOs) under both normoxic and hypoxic conditions (Figure 1) [44]. For example, a recent study by Bryan *et al* demonstrates that steady state concentrations of tissue nitrite and nitroso species are affected by changes in dietary nitrite intake [23]. However, nitrite reduction to NO is very effectively inhibited by oxygen [23, 24], thereby leading to the

fundamental question as to how effective nitrite reduction is along the physiological oxygen gradient. Nonetheless, research clearly demonstrates the enriching dietary intake of nitrite results in significantly less injury from heart attack [1].

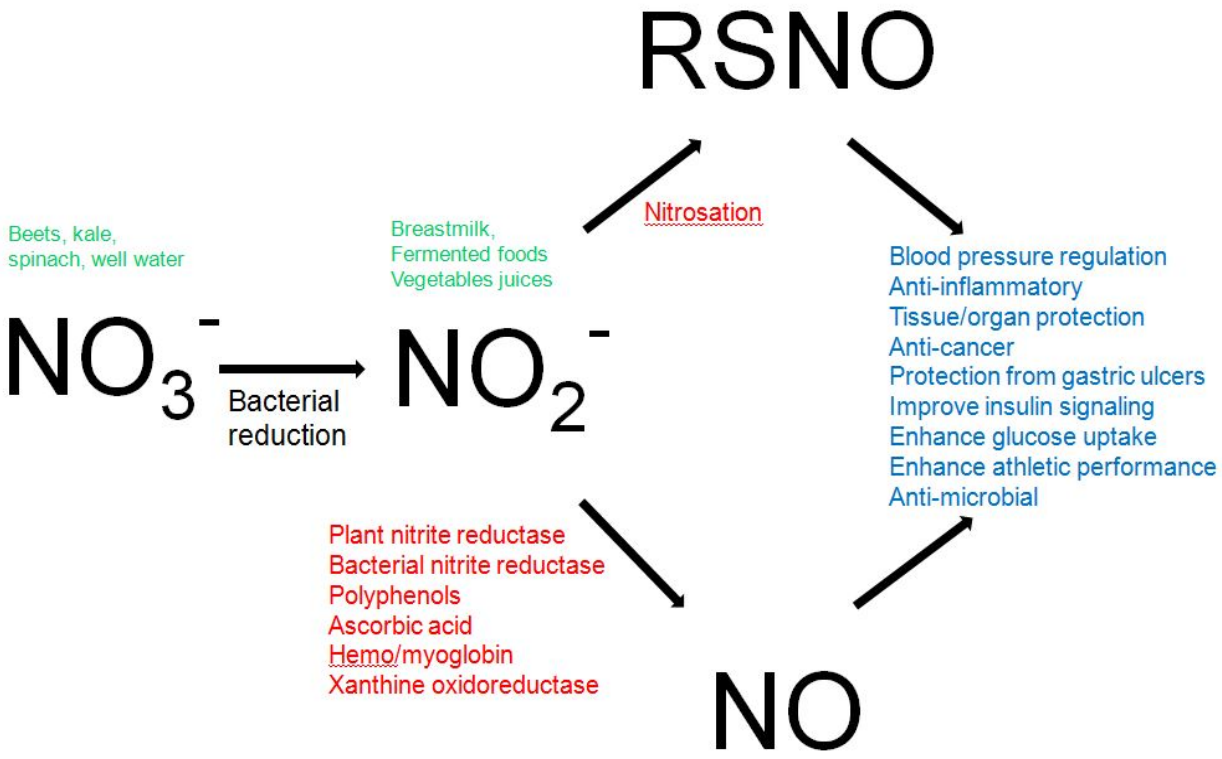

Figure 1: Nitrite is formed from nitrate through bacterial metabolism and can be found naturally in certain foods. Nitrite can form S-nitrosothiols (RSNO) and can be reduced to nitric oxide (NO). Both NO and RSNO have been shown to normalize blood pressure and exert a number of health promoting and disease preventing activities.

Published studies have also demonstrated how nitrite therapy given intravenously prior to reperfusion can protect against hepatic and myocardial ischemia/reperfusion (I/R) injury [51]. Additionally, experiments in primates have revealed a beneficial effect of long-term application of nitrite on cerebral vasospasm [52]. Moreover, inhalation of nebulized nitrite selectively dilates the pulmonary circulation under hypoxic conditions *in vivo* in sheep [53]. Topical application of nitrite improves skin infections and ulcerations [54]. As in meat products, the oxidation of lipids is prevented by nitrite in humans, primarily by NO binding to heme proteins, lowering the amount of free iron available to catalyze initiation and propagation steps of lipid oxidation. Studies by Carr and Frie [55] have demonstrated that myeloperoxidase mediated low density lipid modification is inhibited by nitrite. Furthermore, in the stomach nitrite-derived NO appears to play an important role in host defense [56] and the regulation of gastric mucosal integrity [57]. Nitrite has also been shown to completely prevent microvascular inflammation and endothelial dysfunction resulting from a high fat, high cholesterol diet [6] and reduce C-reactive protein. Chronic sodium nitrite therapy augments ischemia-induced angiogenesis and arteriogenesis, thereby demonstrating that sodium nitrite therapy is a recently discovered therapeutic treatment

for peripheral artery disease and critical limb ischemia [58]. Most recently, a dietary supplement formulated with dietary nitrite and natural product chemistry to reduce nitrite to NO has been shown to modify cardiovascular risk in patients over the age of 40, reduce blood pressure and reduce markers of inflammation [59]. All of these studies, alongside the observation that nitrite can act as a marker of NOS activity [31] opened a new avenue for the diagnostic and therapeutic application of nitrite, especially in cardiovascular diseases, using nitrite as marker as well as an active agent. Accordingly, a report by Kleinbongard *et al.* [60] demonstrates that plasma nitrite levels progressively decrease with increasing cardiovascular risk. Since a substantial portion of steady state nitrite concentrations in blood and tissue are derived from dietary sources [23], modulation of nitrite intake may provide a first line of defense for conditions associated with NO insufficiency [46].

Toxicity of Nitrite and Nitrate: Historically there are three measures of safety and toxicity regarding nitrite. Those are methemoglobinemia, hypotension, and formation of N-nitrosamines. Acute toxicity is defined by methemoglobinemia. The fatal dose of nitrite due to methemoglobinemia is in the range of 22-23 mg/kg body weight (from USFDA Generally Recognized as Safe Food Ingredient: Nitrates and Nitrites (Including Nitrosamines) 1972 by Battele-Columbus Laboratories and Department of Commence, Springfield VA). This dose is approximately 150 times higher than doses which have been used therapeutically in humans. Lower doses of either nitrite or nitrate have caused acute methemoglobinemia, particularly in infants where a high nitrite or nitrate intake has been associated with "blue baby syndrome" caused by methemoglobinemia [61-63]. Although infants are more susceptible to methemoglobinemia, due to the reduced activity of methemoglobin reductase [64] in adults weights of 70-80kg, the amount of nitrite needed for acute toxicity is 1650mg.Therefore, only extremely high concentrations of nitrite can cause methemoglobinemia.

Hypotension or low blood pressure is another potential safety concern with nitrite exposure. However, nitrite is a very poor vasodilator especially at doses that are used to recapitulate NO based signaling [65-67]. Interestingly, nitrite appears to only lower blood pressure in subjects with an elevation in blood pressure [68]. As a result, this creates safe and effective levels for therapy with the mitigation of side effects or unwanted symptoms.

N-Nitrosamines are potent carcinogens and can be formed from nitrite in the presence of low molecular weight amines. Thus, the public health concerns are actually related to the formation of carcinogenic N-Nitrosamines rather than to the nitrite itself. About 30 different animal species are responsive to the carcinogenic effects of approximately 300 different N-nitrosamines [69]. Nitrite is not the only source of N-nitrosamines; exposure can occur in occupational settings, through one's diet, cosmetics, tobacco products, and agricultural chemicals. N-nitrosamine formation in cured meat products occurs when amines react with nitrogen oxides such as nitrite in an acid environment (i.e. the stomach) or when heated to very high temperatures (i.e. bacon frying).

During an infection or inflammation, products of nitric oxide including nitrite are generated that react with the amines by nitrosation reactions to form N-nitrosamines, thereby causing human exposure to N-nitrosamines and possibly linking chronic inflammation to certain cancers. The nitrosation of amines in the body can also be catalyzed by certain bacteria with nitrite to form the N-nitrosamines. The carcinogenicity of N-nitrosamines is due to a very critical

cytochrome P450- mediated metabolic activation step. Although there is evidence to support a plausible biological mechanism for formation of N-nitrosamines, there are also numerous effective inhibitors of N-nitrosation reactions in biological systems [70]. It was discovered that ascorbic acid (vitamin C) very potently inhibits N-nitrosamine formation [71]. Another antioxidant, alpha-tocopherol (vitamin E), has also been shown to inhibit N-nitrosamine formation [72]. Ascorbic acid, erythorbic acid and alpha-tocopherol inhibit N-nitrosamine formation due to their oxidation-reduction properties. For example, when ascorbic acid is oxidized to dehydroascorbic acid, nitrous anhydride, a potent nitrosating agent formed from sodium nitrite, is reduced to NO, which is not a nitrosating agent. Stoichiometrically, one molecule of ascorbic acid can reduce two molecules of acidified nitrite to NO [73, 74]. However, in the presence of dissolved oxygen, NO can be oxidized back to nitrite/nitrous acid. This recycling means that more than half the molar equivalent of ascorbic acid compared to nitrite is required to prevent formation of *N*-nitroso compounds. In other words, for every mole of nitrite, one mole of ascorbate is needed to yield one mole of nitric oxide, plus another 0.5 mole ascorbate to prevent the back reaction. The ratio of ascorbic acid to nitrite is recognized as a major determinant of the generation of N-nitroso compounds within the acidic lumen of the stomach [73]. Contemporary meat-curing methods use ascorbic acid or erythorbate to prevent N-nitrosation reactions and to facilitate the curing process. Furthermore, any vegetable based nitrite or nitrate source typically has sufficient polyphenols or vitamin C to naturally prevent nitrosative chemistry. This is the inherent protective nature of nitrite from natural sources.

CONCLUSION

The emerging health benefits of nitrite represent a profound change in the established paradigm which has existed for the past 50 years. Until the present, scientists have operated under the paradigm of the L-arginine-NO pathway by NOS enzymes as the only pathway to produce NO. There are a number of recycling pathways to regenerate NO from dietary nitrite. The emergence of a redundant pathway for maintenance of NO homeostasis by dietary nitrite provides a new mode of intervention and a new paradigm for restoring NO homeostasis. Nitrite therapy or supplementation may restore NO homeostasis from endothelial dysfunction and provide benefits for a number of diseases characterized by NO insufficiency [13, 75]. If so, this will provide the basis for new preventive or therapeutic strategies and new dietary guidelines for optimal health. There are currently a number of clinical trials using sodium nitrite as a therapeutic agent (please view the following link for more information: www.clinicaltrials.gov). From a public health perspective, we may be able to make better recommendations on diet and dramatically affect the incidence and severity of cardiovascular disease, in addition to subsequent clinical events. Replenishing nitrite through dietary means may then act as a protective measure to compensate for insufficient NOS activity under conditions of hypoxia or in a number of conditions characterized by NO insufficiency. Studies using a patented formulation (US patents 8,303,995, 8,298,589, 8,435,5708 8,962,038 9,119,823 & 9,241,999) in the form of an orally disintegrating tablet found that it could modify cardiovascular risk factors in patients over the age of 40, significantly reduce triglycerides, and reduce blood pressure [59]. This same lozenge was used in a pediatric patient with argininosuccinic aciduria and significantly reduced his blood pressure when prescription medications were ineffective [76]. A more recent clinical trial using the nitrite lozenge reveals that a single lozenge can significantly reduce blood pressure, dilate

blood vessels, improve endothelial function and arterial compliance within hypertensive patients [68]. Furthermore, in a study of pre-hypertensive patients (BP >120/80 < 139/89), administration of one lozenge twice daily led to a significant reduction in blood pressure (12 mmHg systolic and 6 mmHg diastolic) after 30 days [77]. Most recently, this nitrite lozenge was also demonstrated to reduce carotid plague by 11% over six months [78]. Although it appears this dietary supplement is safe and effective in short term studies, long term studies are needed. There are a host of diseases which are associated with decreased NO availability as measured by nitrite. The enormous benefit of exogenous dietary nitrite is becoming more evident in a number of disease models, in both animals and humans. The active agent of some medicinal foods may indeed be nitrite, as nitrite is present in many green leafy vegetables, breast milk, beet root, and many other foods that have shown to be protective from many diseases. The historical classification of nitrite as a "cure" may now have new meaning.

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