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Hypothesis article

Cardioprotective effects of vegetables: Is nitrate the answer?

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Abstract

A diet rich in fruits and vegetables is associated with a lower risk of certain forms of cancer and cardiovascular disease, but the mechanisms behind this protection are not completely understood. Recent epidemiological studies suggest a cardioprotective action afforded specifically by green leafy vegetables. We here propose that these beneficial effects are related to the high content of inorganic nitrate, which in concert with symbiotic bacteria in the oral cavity is converted into nitrite, nitric oxide, and secondary reaction products with vasodilating and tissue-protective properties.

Keywords: Nitric oxide; Nitrite; Cardiovascular; Nitrate reductase; Hypertension; Gastric cancer

Introduction

Our diet exerts important long-term effects on vital body functions and thereby makes an important contribution to health and disease. While high intake of cholesterol, saturated fat, salt, and sugar are generally associated with a greater risk for cardiovascular disease conventional wisdom has it that the opposite is true for abundant consumption of fruits and vegetables [1]. However, surprisingly few studies have evaluated the relationship between fruit and vegetable intake and cardiovascular disease. Recently, a number of large epidemiological studies have attempted to address this issue [1–4]. Joshipura et al. [3,5] found that a high intake of fruits and vegetables was indeed associated with a reduced risk for coronary heart disease and ischemic stroke. The large study population also allowed for analysis of the protection afforded by specific types of foods, and the strongest protection against coronary heart disease was seen with high intake of green leafy vegetables. In another study, Appel et al. [6] looked at the effects of dietary supplementation with vegetables on blood pressure in subjects with borderline hypertension. They found that intake of vegetables decreased blood pressure almost to the same extent as monotherapy with a standard antihypertensive drug. The specific nature of the active constituent(s) responsible for the cardioprotective effects of vegetables is still unknown although fiber, minerals, and antioxidants have all been proposed as viable candidates [1,2]. In the midst of the current hype about the possible significance of polyphenolic antioxidants in protecting organs from the sequelae of oxidative stress, we here wish to put forward an alternative and disarmingly simple hypothesis: We propose that the high content of inorganic nitrate is a major factor contributing to the positive health effects of certain vegetables via bioconversion to nitrite, nitric oxide (NO), and other secondary reaction products (nitroso/nitrosyl compounds), all of which may exert protective effects on the cardiovascular system.

An alternative pathway for NO generation

Continuous generation of NO is essential for the integrity of the cardiovascular system, and a decreased production

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and/or bioavailability of NO is central to the pathogenesis of cardiovascular disorders including atherosclerosis, hypertension, and ischemic heart disease [7,8]. The classical pathway for generation of NO in mammals is via NO synthases present, for example, in the vascular endothelium. These enzymes produce NO from the precursor amino acid, L-arginine, and molecular oxygen. More recently, a fundamentally different pathway for NO generation was discovered in humans [9–11] that occurs via simple reduction of nitrite NO_2^-, a reaction that does not involve NO synthases. This finding was highly surprising since the general belief had been that both nitrate and nitrite are biologically inert waste products from the oxidation of endogenous NO. It is now clear that several alternative routes exist for the in vivo generation of NO from nitrite [12–14]. These include reduction of nitrite by deoxyhemoglobin in blood [15] and reaction with xanthine oxidoreductase [16], enzymes of the mitochondrial respiratory chain [17], and a yet unknown heme- and thiol-containing enzyme activity [18] in tissues. Even vitamin C and simple protons can catalyze this reaction [12,19]. Interestingly, nitrite reduction to NO is greatly enhanced during hypoxia/ischemia, conditions under which the oxygen-dependent L-arginine/NO synthase pathway is malfunctioning [13]. To this end, nitrite reduction can be regarded as a back-up system for the generation of NO in situations of limited oxygen availability. These findings may also have important therapeutic implications since nitrite is a product of the metabolic breakdown of organic nitrates (such as the antianginal drug nitroglycerin) in tissues, where it arises in amounts far higher than those for NO and related nitroso/nitrosyl species [20].

Nitrite protects the cardiovascular system

While the notion that nitrite and nitrate may be beneficial rather than detrimental to human health is not entirely new [9,10,21–24], much of these discussions evolved around direct antimicrobial effects of acidified nitrite in the GI tract and on the skin. Although long-term toxicological studies in rats have not confirmed that nitrite or nitrate are carcinogenic, and epidemiological studies have failed to provide a causal link between nitrate intake and cancer [25,26], these considerations have not diminished concerns of the broader public and public health authorities about current levels in drinking water and food. During the past few years, however, an impressive amount of new data supporting a role for nitrite in the regulation of cardiovascular function appeared in the literature. Nitrite is now emerging as a physiological regulator of hypoxic vasodilation and mitochondrial respiration, and also a modulator of ischemia-reperfusion tissue injury and infarction [13,14,27]. Cosby et al. [15] showed that infusion of nitrite can cause vasodilation in humans and suggested a role for nitrite in blood flow regulation. They furthermore suggested a role for deoxyhemoglobin in intravascular conversion of nitrite to NO. Duranski et al. [28] studied the cytoprotective effects of nitrite in an animal model of cardiac and hepatic ischemia. By treating mice with low doses of sodium nitrite systemically, they could reduce infarct size dramatically. In higher doses, nitrite can prevent delayed cerebral vasospasm after subarachnoid hemorrhage [29], and attenuate pulmonary hypertension when inhaled [30]. While most studies on nitrite appear to show that its biological effects occur via generation of NO, one very recent study indicates that nitrite may act as a signaling molecule in its own right [18]. Naturally, the substrate nitrite needs to be readily available for this system to operate, which requires at least one available source and an effective uptake and transport system. In humans, there are two large sources of nitrite [31]. One is endogenous formation of NO, which is spontaneously oxidized in tissues and blood to form nitrite, and the second is the diet. In the latter, it exists largely in the form of the precursor nitrate. Only a minor portion is taken up directly as nitrite via ingestion of, e.g., cured meats such as bacon and sausages where it serves, often in conjunction with vitamin C, as a food preservative.

Bioconversion of dietary nitrate to nitrite and NO

Nitrate has been used for food preservation purposes since centuries. Largely out of safety concerns in relation to the formation of potentially carcinogenic nitrosamines and the formation of methemoglobin in infants these days maximally allowable concentrations of nitrite and nitrate in drinking water are strictly regulated in Europe, the US, and many other countries. However, the by far dominating dietary source (>80%) of nitrate is the ingestion of vegetables [31]. Green leafy vegetables such as spinach and lettuce, but also cauliflower and celery, are especially rich in nitrate as are strawberries, grapes, and a few other fruits [32] (Fig. 1). Total intake of nitrate is subject to seasonal variations, fertilizer use and cooking procedures, and varies greatly between individuals and regions. Vegetarians consume up to 10 times more nitrate than non-vegetarians, and a typical Mediterranean diet, for example, is likely to contain considerably more nitrate than the average Western diet.

Fig. 1. Spinach, a prototypical green leafy vegetable rich in nitrate, originates from Persia from where it spread to Europe around 1300 and to North America by the early part of the 19th century. More recently, it has been popularized by the cartoon character "Popeye," who attributes his amazing strength to a daily diet of this vegetable.

Ingested nitrate is rapidly absorbed in the small intestine and readily distributed throughout the body via the circulation. For yet unknown reasons as much as 25% is actively taken up from the blood by the salivary glands to be excreted in the saliva [33]. A substantial portion (~20%) of this nitrate is then reduced to nitrite by commensal bacteria in the oral cavity [33]. These facultative anaerobes use nitrate as an alternative electron acceptor to produce energy. Without the enterosalivary circulation of nitrate and the oral microflora, nitrate would leave the body unmodified as this chemically stable anion cannot be metabolized by mammalian enzymes. We could recently show that plasma levels of nitrite increase greatly after an oral load of sodium nitrate in an amount corresponding to about 300 g of spinach [34]. The increase in plasma nitrite was completely prevented if the test individuals avoided swallowing for a certain period after nitrate intake thereby illustrating its enterosalivary origin. It is intriguing to compare the systemic nitrite load provided by a nitrate-rich meal with the amount of nitrite needed to protect tissues in the setting of ischemia-reperfusion. In those studies, the maximal protective effects of exogenous nitrite are seen already at a very modest dose [28]. In fact, a similar or even higher systemic load of nitrite is achieved by ingestion of no more than 100 g of lettuce or spinach. A recent animal study by Bryan et al. further supports a role for dietary nitrate in the regulation of cardiovascular function. In this study, we could show that by limiting the intake of nitrate and nitrite with the diet, the tissue levels of nitrite were effectively depleted within 2 days and these changes were accompanied by a concomitant decrease in signaling pathways typically ascribed to be triggered by NO (i.e., the depletion in tissue nitrite resulted in a measurable reduction in cGMP). Taken together, these data suggest that dietary-derived nitrate can be converted into bioactive nitrite and NO in amounts sufficient to have profound effects on the cardiovascular system.

It is possible that other constituent of our diet may work in concert to enhance the beneficial effects of nitrate. One such example is vitamin C which is abundant in many fruits and vegetables. Interestingly, this vitamin greatly enhances NO generation from nitrite [12,35]. Another intriguing possibility is the reaction between nitrite/NO and unsaturated fatty acids (FAs, e.g., linoleic and oleic acid) which then may become nitrated (NO_2-FA) [36]. Recent studies by Freeman and co-workers [37,38] elegantly show that nitrated FAs possess antiinflammatory activity in vitro which may theoretically be of importance in protection against cardiovascular diseases such as atherosclerosis. The gastric milieu seems ideal for generation of nitrated FAs as the combination of nitrite and acid enhances nitration reactions.

Testing the hypothesis

A combination of experimental studies and clinical trials will allow us to determine if dietary nitrate does indeed provide the purported protection against cardiovascular disease. Several animal models of cardiovascular disease may readily lend themselves to intervention studies with nitrate. In this context, the use of germ-free animals may prove very useful. In theory, such animals should have no benefit from nitrate supplementation as they cannot convert dietary nitrate into bioactive nitrogen oxides [39]. Animal studies will also be useful to find the optimal dose of nitrate needed for cardioprotection. There is a possibility that the beneficial effects of nitrate are lost if the intake is too high. In patients, the physiological and biochemical aspects of cardiovascular function could be easily evaluated by the measurement of, e.g., endothelial function in combination with dietary manipulations, either by comparing individuals with a regular and a low content of nitrite/nitrate or by simple supplementation with nitrate. In such investigations, it is important to recognize and avoid possible confounding factors such as the nitrate content of the drinking water, vitamin supplementation, smoking habits, and other factors. Further insight could be provided by epidemiologic studies comparing cardiovascular disease burden with nitrate intake. Since vegetables contain many other constituents with potential cardioprotective properties, the predictive power of such epidemiological studies is limited and fails to prove causality. Thus, large prospective trials with supplementation of inorganic nitrate on top of a standard diet will be necessary to unequivocally test the validity of our hypothesis.

Conclusion

We here propose that the protective effect of certain vegetables on the cardiovascular system is related to their high content of nitrate. The mechanism involves reduction of dietary nitrate to nitrite, nitric oxide, and possibly other biologically active reaction products in a process that requires cooperation with symbiotic bacteria in the oral cavity. A continuous intake of nitrate-containing food such as green leafy vegetables may ensure that tissue levels of NO and other nitrogen/nitrosyl species are maintained at a level sufficient to compensate for any disturbances in endogenous NO synthesis. Naturally, this provocative hypothesis needs to be carefully tested in clinical trials. If proven true, however, these considerations could have a profound impact on our view of the role of diet and commensal bacteria in the regulation of normal physiological processes and prevention of cardiovascular disease.

Conflict of interest statement

M.F. is a paid consultant and member of the Scientific Advisory Board of Nitromed, Inc.

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