

Chewing Well During Meals May Benefit Health Via the Enterosalivary Nitrate–Nitrite–Nitric Oxide Pathway

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ABSTRACT

Saliva contains nitrates recycled from ingested foods (e.g., vegetables) via the enterosalivary pathway. During meals, thorough chewing enhances salivary flow and the reduction of salivary nitrate to nitrite by oral commensal bacteria and thus leads to a nitrite-rich gastric environment. Subsequently, nitric oxide (NO) and *S*-nitrosothiols, rather than *N*-nitroso compounds, are generated in the acidic stomach. These molecules may confer gastric and systemic health benefits by transducing NO-mediated signals, particularly in patients with vascular endothelial dysfunction and

metabolic syndrome, which is associated with reduced endogenous NO generation consequent to impaired NO synthase activity. This article reviews literature suggesting that the thorough chewing of food produces a nitrite-rich gastric environment and promotes health benefits by enhancing NO bioavailability via the enterosalivary nitrate-nitrite-NO pathway.

Key words: Chewing; Saliva; nitric oxide; enterosalivary nitrate-nitrite-NO pathway; *S*-nitrosothiols; *N*-nitroso compounds

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INTRODUCTION

More thorough chewing of food increases the secretion of saliva into the oral cavity, which improves not only food digestion but also oral health maintenance^[1]. Recently, high salivary nitrate levels via the enterosalivary pathway have elicited research attention from the viewpoint of the beneficial effects of nitric oxide (NO) on health and disease^[2]. In Japan, the “Yojo-kun,” a historical document written by the Japanese Confucian and herbalist Ekiken Kaibara (1630-1714), appears to be a self-help or rule-of-thumb guide on good health and an increasing lifespan^[3]. Kaibara lived to an age of 84 years during the Edo period, when the average life expectancy in Japan was approximately 40-50 years, possibly by practicing the concepts in his book. The Yojo-kun contains advice regarding saliva, such as “do not spit, feed internal organs by swallowing well-chewed food mixed with saliva.” Although dated, Kaibara’s recommendations may be applicable to modern preventive medicine, particularly the prevention of a variety of lifestyle-related diseases by enhancing NO bioavailability via the enterosalivary nitrate-nitrite-NO pathway^[2]. This review aims to verify the health benefits, rather than the carcinogenicity, of nitrite-rich saliva stimulated by thorough chewing, with support from the literature.

THE ENTEROSALIVARY NITRATE-NITRITE-NO PATHWAY

Why do we chew our food? Chewing mechanically tears food into smaller pieces to facilitate swallowing and stimulate the secretion of saliva, which contains the enzymes necessary for food digestion and subsequent intestinal absorption. Chewing also activates mechanoreceptors by compressing the teeth into the periodontal membrane, which transmits impulses via the trigeminal nerve to the salivation center^[1]. The salivary flow rate varies widely depending on age, sex, food type, and chewing force and frequency, with average rates of 0.3 and 1.5 mL/min under non-stimulated and chewing-stimulated conditions, respectively^[1]. The hardness and size of the chewed object and the chewing force exerted by the engaged muscles correlate positively with the salivary flow rate^[1,4].

Saliva contains a high concentration of nitrate because dietary nitrate (mainly from vegetables), along with endogenous nitrate produced via the oxidation of endogenous NO, is absorbed from the upper intestine into systemic circulation. Approximately 25% of the nitrate in the bloodstream enters the oral cavity along with saliva from the salivary glands (Figure 1)^[5]. The reduction of nitrate to nitrite by oral bacteria in the saliva increases in the presence of

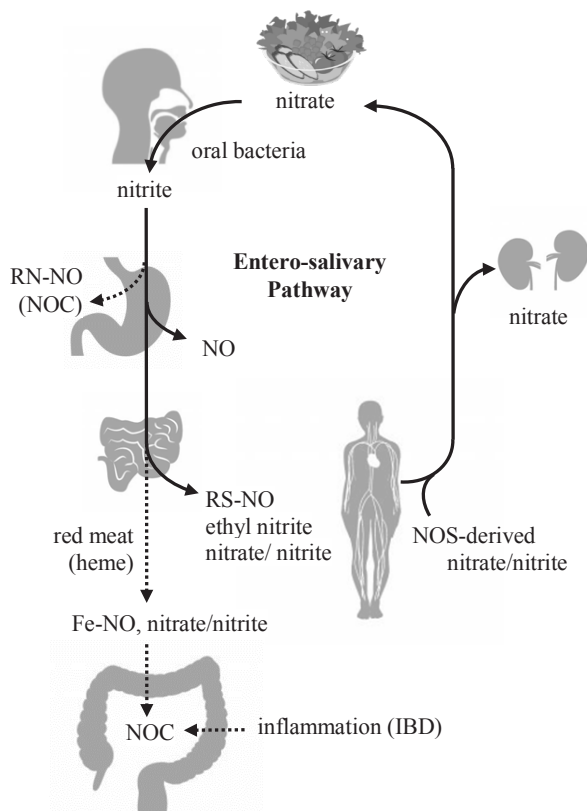


Figure 1 The enterosalivary nitrate–nitrite–nitric oxide (NO) pathway. Saliva contains a high concentration of nitrates, which are derived largely from green leafy vegetables and processed via the enterosalivary pathway. Salivary nitrate is reduced to nitrite in the oral cavity, resulting in a nitrite-rich gastric environment that may be beneficial or carcinogenic, depending on gastric acidity and diet. NO and reactive nitrogen species, including RSNOs, promote local and systemic health by enhancing NO bioavailability. However, in cases of low stomach acidity and high red and processed meat intake, nitrite and heme protein-associated NO enters the lower intestine and may induce carcinogenic NOC formation in the colon. RSNOs: S-nitrosothiols, RN-NO (NOC): N-nitroso compound, Fe-NO: heme (nitrosyl) iron complex, NOS: NO synthase, IBD: inflammatory bowel disease

chewing^[6]. Chewing also promotes contact between salivary nitrate and the tongue, where bacteria-mediated nitrate reduction occurs. Hence, the nitrite/nitrate ratio increases in proportion to the duration and frequency of chewing^[4]. The removal of oral commensal bacteria with antibacterial mouthwash attenuates the conversion of oral nitrate to nitrite and the subsequent increase in plasma nitrite levels after dietary nitrate consumption, while also eliminating the hypotensive effect of dietary nitrate on the vascular system^[7]. Plasma nitrite is actively transported from circulation and stored in tissues or organs such as the heart, blood vessels, liver, and muscles to show NO bioavailability by reducing tissue nitrite to NO via several enzymatic and non-enzymatic processes as needed and by protecting organs from ischemia and reperfusion injury^[2].

Chewing causes more nitrite to enter the acid-rich stomach, where it is protonated to form nitrous acid. This acid can decompose spontaneously to NO and related compounds that exhibit nitrosylating and nitrosating properties^[2]. In the stomach, short-lived NO has local benefits; for example, it increases mucosal blood flow and subsequent mucus secretion and protects against swallowed bacteria, which multiply upon the concomitant intake of antioxidant vitamins and polyphenols^[2]. The nitrite-rich stomach also promotes the S-nitrosation of not only gastric membrane-associated and secreted proteins (e.g., cysteine-rich mucus glycoproteins) that protect gastric mucosa^[8], but also of thiol compounds in some diets and drugs^[9]. The combined (but not separate) intake of thiol compounds and dietary nitrite may promote the generation of S-nitrosothiols (RSNOs) in the stomach and increased plasma levels of S-nitroso adducts to proteins and drugs (e.g., albumin and clopidogrel)^[9,10,11], as well as the subsequent transnitrosation of NO groups to small-molecule thiols, which form stable S-nitroso-glutathione and S-nitroso-cysteine molecules. This is a principal mechanism by which NO-mediated signals are transduced to modulate various functions, including the activities of transcription factors, enzymes, membrane receptors, and ion channels, in cyclic GMP-dependent and -independent manners^[12]. Salivary nitrite may thus constitute an important part of the enterosalivary nitrate-nitrite-NO pathway for health promotion (Figure 1).

IS NITRITE-RICH SALIVA HARMFUL?

Previous animal experiments implicated dietary nitrite in the formation of carcinogenic nitrosamine^[13]. Consequently, nitrate and nitrite levels are restricted in food and drinking water intended for humans^[14]. Chronic exposure to nitrates in food and drinking water has been linked to an increased risk of colon cancer in a limited population with a low vitamin C^[15] and high red meat^[16] intake. However, the World Cancer Research Fund Continuous Update Report in 2015 reported no consistent epidemiological evidence of an increased risk of human cancer due to high nitrate consumption^[17].

Most dietary nitrite is derived from salivary nitrate via enterosalivary recycling^[14]. Therefore, if salivary nitrite were carcinogenic in the GI tract, we would need to spit continuously. This scenario is difficult to imagine. When assessing the causal relationship of dietary nitrate/nitrite with carcinogenesis, diets and gut environments should be considered for the following reasons. First, nitrosonium ions, which are formed via salivary nitrite protonation in the acidic stomach, kinetically favor the generation of RSNOs rather than N-nitrosamines by binding to dietary protein thiols via nucleophilic attractions to sulfur atoms but not the nitrogen atoms of amines^[14]. However, achlorhydria, a consequence of prolonged proton pump inhibitor use and *Helicobacter pylori*-induced chronic gastritis, is associated with the formation of N-nitrosamines rather than RSNOs. Gastric acid-

ity, therefore, is a key determinant of whether dietary nitrite will be converted to beneficial versus carcinogenic nitroso compounds in the stomach.

Second, most dietary nitrite is absorbed by the upper intestine and does not reach the lower intestine. However, higher fecal nitrite levels may be observed, particularly in people with a high intake of red meat versus those with diets rich in white meat, fish, and vegetables^[18], suggesting that heme protein may support nitrosating activity in the lower GI tract. In 2015, the International Agency for Research on Cancer (IARC) classified red and heme protein-rich processed meats as carcinogenic to humans (Group 2A and Group 1, respectively)^[19] because they stimulate lipid peroxidation and the subsequent generation of free radicals, as well as carcinogenic *N*-nitroso compounds (NOCs), in the lower GI tract^[20]. Initially, the formation of RSNOs at heme cysteine residues occurs in the acidic stomach. These RSNOs become unstable in the upper intestine, after which heme iron is nitrosylated (nitrosyl heme: Fe-NO) via salvage of the released NO from cysteine residues during passing through the reductive and anaerobic lower intestine. Although this nitrosyl heme may be directly responsible for colonic NOC formation even in the presence of minimal microbial flora, close contact between fecal nitrosyl heme and oxygen diffused from the enteral capillary vasculature may induce the formation of nitrite. This can be subsequently reduced by nitrite reductases to form NOCs with nitrosatable amines, a process mediated by facultative anaerobes (e.g., *Escherichia coli* and *Proteus morgana*, particularly in dysbiotic microbial flora) that reside in the aerobic inner mucous layer of the colon and use nitrate and nitrite for respiratory denitrification^[18].

Hypoxemia due to methemoglobinemia is also caused by dietary nitrate and nitrite. In the mid-20th century, Fawns and Aldridge described infants with methemoglobinemia (i.e., blue-baby syndrome)^[21]. These cases, however, clearly occurred under special circumstances involving bacteria- and fertilizer-contaminated well water, which had high nitrite levels, for the preparation of formula fed to infants. When absorbed by the blood, the nitrite reacts with hemoglobin to form methemoglobin, which cannot bind oxygen. Although this reaction is reversible, infants have lower levels of methemoglobin reductase activity than adults and are therefore highly susceptible to methemoglobinemia^[22].

If dietary nitrite (including salivary nitrite) is thoroughly catabolized in the acidic stomach and not transferred to the lower intestine, it is not necessarily a carcinogen in the GI tract. Rather, nitrite-rich saliva, a consequence of well-chewed food, helps to maintain health and prevent lifestyle-related diseases, rather than increasing the risk of carcinogenesis.

CONCLUSION

NO participates in a variety of key signaling pathways and is thus essential to life^[23]. Accordingly, this simple molecule has survived evolution and has emerged as one of the most important compounds in the field of life science. In modern society, poor NO availability, a consequence of aging and obesity, is a major cause of lifestyle-related diseases^[2]. As described above, thorough chewing during meals may enhance enterosalivary NO availability by providing a nitrite-rich gastric environment. Well-chewed food may also help to compensate reductions in endogenous NO synthase-derived NO levels, which decline during aging and in metabolic syndromes associated with vascular endothelial dysfunction. Frequent and thorough food chewing is a simple and inexpensive method for enhancing NO bioavailability and extending the lifespan. Lifestyle changes such as a nutritionally balanced diet and physical activity are also recommended.

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