Dietary Nitrate and the Maintenance of Oral Health

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Nitrate (NO_3) is the oxidative product of nitric oxide (NO) and nitrite (NO_2). As soil content, NO_3 is an essential substrate for all plant growth and provides, among other things, the nitrogen required for the synthesis of nitrogenous amino acids. It is taken up by the plant roots and originates either from the microbial decomposition of organic waste or from the activity of certain soil bacteria utilizing atmospheric nitrogen for the synthesis of ammonia, nitrite and nitrate. Since available nitrate is the limiting factor for plant growth, it is deliberately added to the soil in agricultural crop production in the form of nitrate-containing fertilizers. Although the role of the oral microbiota as a key element in the alternative formation of NO_2 and NO from nitrate-rich foods has been known for many years, it is surprisingly only in recent years that the therapeutic and preventive prospects of a nitrate-rich diet have attracted the interest of dental researchers.

Keywords: nitric oxide ; nitrite ; nitrate ; diet ; oral ; periodontitis ; caries

1. Impact of Dietary Nitrate on Caries Development

As already explained, salivary nitrite has a pronounced antibacterial effect that increases with decreasing pH values and easily penetrates microbial biofilms [1]. Increasing the level of nitrite in saliva by eating a diet rich in nitrates has therefore been proposed as a promising alternative strategy to control tooth decay. Scoffield et al. [2] evaluated the impact of nitrite in the culture media of *Streptococcus mutans* co-cultivated with *Streptococcus parasanguinis*. They observed a significant reduction in *S. mutans* growth rate and biofilm formation compared to the controls not being exposed to nitrite. In a subsequent animal study, inoculation of rats with *S. parasanguinis* prior to infection with *S. mutans*, supplemented with the addition of nitrite to the drinking water, resulted in a significantly lower number of newly formed carious lesions in enamel and dentin compared to the findings in the control animals, which did not consume nitrite-supplemented drinking water. These observations are supported by the results of Hohensinn et al. [3], who reported a significant increase in salivary Nitrate (NO_3), nitrite (NO_2) and nitric oxide (NO) levels accompanied by a significant increase in mean salivary pH from pH 7.0 to pH 7.5 in healthy volunteers who regularly consumed a nitrate-rich beetroot juice.

Similarly, Burleigh et al. [4] observed a significant attenuation of salivary pH lowering in male endurance runners following the consumption of carbohydrate-rich snacks by the simultaneous ingestion of nitrate-rich beetroot juice.

More detailed insights into the role of salivary nitrate in promoting the establishment of a eubiotic non-cariogenic oral microbiome were recently provided by Huffines et al. [5]. They analyzed the impact of the addition of nitrite to the culture medium in a mixed in vitro biofilm model containing the commensal *S. parasanguinis*, and the cariogenic pathogens *S. mutans* and *Candida albicans. S. parasanguinis* showed a high degree of intrinsic resistance to the experimental increase in nitrosative stress and even increased it by the formation of peroxide, which in the presence of nitrite is readily converted to peroxynitrite a potent antibacterial compound. *S. mutans* and *C. albicans*, by contrast, were very sensitive to nitrosative stress and showed a significant reduction in growth rate and biofilm formation.

However, there are currently no data from controlled clinical trials to confirm these promising findings on the benefits of a nitrate-rich diet in controlling caries development.

2. Impact of Dietary Nitrate on the Development of Gingivitis and Periodontitis

For many years the focus of research efforts in periodontology was mostly on the role of Inducible Nitric Oxide Synthase (iNOS) activation in periodontal tissue breakdown and the development of therapeutic approaches to control it [6][7]. As described before, salivary nitrite concentration may either reflect the metabolic activity of nitrate-reducing oral bacteria and/or are influenced by a local efflux of iNOS-generated NO and NO_2 from inflamed tissue sites in gingival and periodontal inflammation; therefore, several authors assessed the usefulness of NO and NO_2 level measurement in gingival crevicular fluid and saliva as diagnostic markers of periodontal health with diverging results. Topcu et al. [8]

investigated nitrate and nitrite levels in saliva and gingival fluid and reported significantly increased nitrite levels in the gingival fluid of gingivitis patients compared to healthy controls. In an in vitro study, Hussain et al. [9] exposed cultured oral keratocytes to the culture supernatants of a variety of oral microorganisms, including *Aggregatibacter actinomycetemcomitas*, *Campylobacter rectus*, *Porphyromonas gingivalis, Streptococcus salivarius* and *C. albicans*. They observed significantly different concentrations of nitrite released into the cell culture medium by the keratocytes in response to the exposure to the different bacterial supernatants, with the supernatants of *A. actinomycetemcomitans* and *C. rectus* eliciting the strongest response. The same group of researchers demonstrated in a later study [10] that the number of iNOS and adrenomedullin (AM) expressing cells was three-fold higher in inflamed than in healthy gingival tissues. They also observed that salivary NO and AM levels were significantly higher in patients with aggressive periodontitis than in patients with chronic periodontitis or gingivitis. The mean NO levels in gingival crevicular fluid differed significantly between aggressive periodontitis and chronic periodontitis, as well as between chronic periodontitis and gingivitis. Han et al. [11] showed similar findings in a cohort of periodontitis-affected elderly Koreans. In contrast, Aurer et al. [12] reported decreased salivary levels of NO and NO_2 in periodontitis patients."

Again, only recently the significance of dietary NO_3 consumption for the maintenance of periodontal health has been the subject of scientific investigations. In a controlled clinical intervention trial, Jockel-Schneider et al. [13] assessed the influence of the regular consumption of a nitrate-rich lettuce juice beverage on the manifestation of chronic gingivitis in a cohort of treated periodontitis patients regularly seeking periodontal aftercare. They were able to show that the targeted daily intake of 200 mg of dietary NO_3 over an observation period of two weeks after professional mechanical plaque removal (PMPR) led to a halving of gingival inflammatory expression compared to baseline. By contrast, in controls, having consumed an identical lettuce juice but without any NO_3 content, the ameliorative impact of PMPR on gingival inflammation was only small and not significant when compared to baseline levels. Microbial samples taken from the periodontal pockets of the study participants at the onset and at the end of the study were subjected to whole-genome sequencing at the genus level. In the test group consuming a nitrate-rich diet, a significant shift in the composition of the different bacterial genera was observed. At the end of the study, the frequency of bacterial genera associated with periodontal inflammation such as *Treponema* and *Prevotella* was significantly reduced, while the presence of genera associated with periodontal health such as *Rothia* and *Neisseria* was significantly increased. In control group patients, by contrast, despite being equally subjected to PMPR but having consumed a nitrate-depleted diet, differences in the composition of the microbiota between baseline and reevaluation were only small and not significant [14]. The persistence or rapid recurrence of bacterial dysbiosis in the control group clearly proved that the effect of mechanical plaque removal may only be symptomatic, while the significant resolution of bacterial dysbiosis observed in the test group suggests that dietary aspects may play an important causative role in the overgrowth of disease-promoting oral pathobionts. These data are partially supported by the findings of another clinical investigation. Woelber et al. [15] reported an inhibitory influence of a daily diet rich in omega-3 fatty acids, antioxidants and dietary NO_3 on the manifestation of gingivitis in a cohort of individuals who had been habitually consuming a Western diet containing over 45% processed carbohydrates before the onset of the research; however, due to the combined consumption of various food components with anti-inflammatory properties evaluated in this trial, it is not possible to assess the individual significance and contribution of dietary NO_3 consumption to the results. Nevertheless, despite these promising data on the efficacy of a high-nitrate diet as an adjuvant in preventing and treating periodontal inflammation, the available evidence must be considered preliminary until additional results from larger controlled clinical trials are available.

3. Impact of Oral Antiseptics on Salivary NO_2 Levels

In many industrialized countries, the regular use of an antibacterial mouth rinse is a popular supplement to daily toothbrushing. This may be problematic as an undifferentiated long-term use of oral antiseptics not only inhibits the growths of pathogens but may concomitantly impair the vitality and metabolism of oral symbionts such as the aforementioned nitrate-reducing microbiota on the tongue. Kapil et al. [16] reported that the use of an antiseptic chlorhexidine-based mouthwash suppressed microbial nitrate reduction and resulted in a significant increase in systolic blood pressure of young healthy volunteers. Other authors have confirmed this finding [17], while in epidemiological surveys, the frequent use of mouthwashes was associated with elevated incidences of hypertension [18] and type 2 diabetes [19], as the availability of nitrate ingested with the daily diet has shown to be an important modulator of insulin resistance in obese adults [20] and metabolic syndrome patients [21]; therefore, although the use of proven oral antiseptics such as chlorhexidine is still considered safe in principle, current guidelines for the prevention and treatment of caries and periodontal disease recommend their use as an adjunct to mechanical plaque control only for certain indications and limited time spans [22][23].

References

1. Dykhuizen, R.S.; Frazer, R.; Duncan, C.; Smith, C.C.; Golden, M.; Benjamin, N.; Leifert, C. Antimicrobial effect of acidified nitrite on gut pathogens: Importance of dietary nitrate in host defense. Antimicrob. Agents Chemother. 1996, 40, 1422–1425.
2. Scoffield, J.; Michalek, S.; Harber, G.; Eipers, P.; Morrow, C.; Wu, H. Dietary Nitrite Drives Disease Outcomes in Oral Polymicrobial Infections. J. Dent. Res. 2019, 98, 1020–1026.
3. Hohensinn, B.; Haselgrübler, R.; Müller, U.; Stadlbauer, V.; Lanzerstorfer, P.; Lirk, G.; Höglinger, O.; Weghuber, J. Sustaining elevated levels of nitrite in the oral cavity through consumption of nitrate-rich beetroot juice in young healthy adults reduces salivary pH. Nitric. Oxide. 2016, 60, 10–15.
4. Burleigh, M.C.; Sculthorpe, N.; Henriquez, F.L.; Easton, C. Nitrate-rich beetroot juice offsets salivary acidity following carbohydrate ingestion before and after endurance exercise in healthy male runners. PLoS ONE 2020, 15, e0243755.
5. Huffines, J.T.; Stoner, S.N.; Baty, J.J.; Scoffield, J.A. Nitrite Triggers Reprogramming of the Oral Polymicrobial Metabolome by a Commensal Streptococcus. Front. Cell. Infect. Microbiol. 2022, 12, 833339.
6. Parvu, A.E.; Alb, S.F.; Craciun, A.; Taulescu, M.A. Efficacy of subantimicrobial-dose doxycycline against nitrosative stress in chronic periodontitis. Acta Pharmacol. Sin. 2013, 34, 247–254.
7. Kendall, H.K.; Marshall, R.I.; Bartold, P.M. Nitric oxide and tissue destruction. Oral. Dis. 2001, 7, 2–10.
8. Topcu Ali, O.; Akalin, F.A.; Sahbazoglu, K.B.; Yamalik, N.; Kilinc, K.; Karabulut, E.; Tozum, T.F. Nitrite and nitrate levels of gingival crevicular fluid and saliva in subjects with gingivitis and chronic periodontitis. J. Oral Maxillofac. Res. 2014, 5, e5.
9. Hussain, Q.A.; McKay, I.J.; Gonzales-Marin, C.; Allaker, R.P. Regulation of adrenomedullin and nitric oxide production by periodontal bacteria. J. Periodontal. Res. 2015, 50, 650–657.
10. Hussain, Q.A.; McKay, I.J.; Gonzales-Marin, C.; Allaker, R.P. Detection of adrenomedullin and nitric oxide in different forms of periodontal disease. J. Periodontal. Res. 2016, 51, 16–25.
11. Han, D.H.; Kim, M.S.; Shin, H.S.; Park, K.P.; Kim, H.D. Association between periodontitis and salivary nitric oxide metabolites among community elderly Koreans. J. Periodontol. 2013, 84, 776–784.
12. Aurer, A.; Aleksić, J.; Ivić-Kardum, M.; Aurer, J.; Culo, F. Nitric oxide synthesis is decreased in periodontitis. J. Clin. Periodontol. 2001, 28, 565–568.
13. Jockel-Schneider, Y.; Gossner, S.K.; Petersen, N.; Stolzel, P.; Hagele, F.; Schweiggert, R.M.; Haubitz, I.; Eigenthaler, M.; Carle, R.; Schlagenhauf, U. Stimulation of the nitrate-nitrite-NO-metabolism by repeated lettuce juice consumption decreases gingival inflammation in periodontal recall patients: A randomized, double-blinded, placebo-controlled clinical trial. J. Clin. Periodontol. 2016, 43, 603–608.
14. Jockel-Schneider, Y.; Schlagenhauf, U.; Stolzel, P.; Gossner, S.; Carle, R.; Ehmke, B.; Prior, K.; Hagenfeld, D. Nitrate-rich diet alters the composition of the oral microbiota in periodontal recall patients. J. Periodontol. 2021, 92, 1536–1545.
15. Woelber, J.P.; Gartner, M.; Breuninger, L.; Anderson, A.; Konig, D.; Hellwig, E.; Al-Ahmad, A.; Vach, K.; Dotsch, A.; Ratka-Kruger, P.; et al. The influence of an anti-inflammatory diet on gingivitis. A randomized controlled trial. J. Clin. Periodontol. 2019, 46, 481–490.
16. Kapil, V.; Haydar, S.M.; Pearl, V.; Lundberg, J.O.; Weitzberg, E.; Ahluwalia, A. Physiological role for nitrate-reducing oral bacteria in blood pressure control. Free Radic. Biol. Med. 2013, 55, 93–100.
17. Bondonno, C.P.; Liu, A.H.; Croft, K.D.; Considine, M.J.; Puddey, I.B.; Woodman, R.J.; Hodgson, J.M. Antibacterial mouthwash blunts oral nitrate reduction and increases blood pressure in treated hypertensive men and women. Am. J. Hypertens. 2015, 28, 572–575.
18. Joshipura, K.; Munoz-Torres, F.; Fernandez-Santiago, J.; Patel, R.P.; Lopez-Candales, A. Over-the-counter mouthwash use, nitric oxide and hypertension risk. Blood Press. 2020, 29, 103–112.
19. Joshipura, K.J.; Munoz-Torres, F.J.; Morou-Bermudez, E.; Patel, R.P. Over-the-counter mouthwash use and risk of pre-diabetes/diabetes. Nitric Oxide 2017, 1, 14–20.
20. Beals, J.W.; Binns, S.E.; Davis, J.L.; Giordano, G.R.; Klochak, A.L.; Paris, H.L.; Schweder, M.M.; Peltonen, G.L.; Scalzo, R.L.; Bell, C. Concurrent Beet Juice and Carbohydrate Ingestion: Influence on Glucose Tolerance in Obese and Nonobese Adults. J. Nutr. Metab. 2017, 2017, 6436783.
21. Hughan, K.S.; Levine, A.; Helbling, N.; Anthony, S.; DeLany, J.P.; Stefanovic-Racic, M.; Goodpaster, B.H.; Gladwin, M.T. Effects of Oral Sodium Nitrite on Blood Pressure, Insulin Sensitivity, and Intima-Media Arterial Thickening in Adults With Hypertension and Metabolic Syndrome. Hypertension 2020, 76, 866–874.

22. Paris, S.; Banerjee, A.; Bottenberg, P.; Breschi, L.; Campus, G.; Domejean, S.; Ekstrand, K.; Giacaman, R.A.; Haak, R.; Hannig, M.; et al. How to Intervene in the Caries Process in Older Adults: A Joint ORCA and EFCD Expert Delphi Consensus Statement. Caries Res. 2020, 54, 459–465.
23. Sanz, M.; Herrera, D.; Kebschull, M.; Chapple, I.; Jepsen, S.; Beglundh, T.; Sculean, A.; Tonetti, M.S.; Participants, E.F.P.W.; Methodological, C. Treatment of stage I-III periodontitis-The EFP S3 level clinical practice guideline. J. Clin. Periodontol. 2020, 47 (Suppl. 22), 4–60.