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Nitrate and body homeostasis

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ABSTRACT

Homeostasis is essential for the maintenance of health, and how it is maintained is the focus of attention in homeostatic medicine. As a natural dietary nutrient, nitrate is critical in regulating homeostasis. This article summarizes nitrate's cognitive history, sources, and metabolism. It discusses the relationship and possible mechanism between nitrate and organism homeostasis from three aspects: flora homeostasis, inflammation-immunity homeostasis, and energy metabolism homeostasis, as well as the clinical application scenarios of nitrate as a promising new generation of drugs, including preventing and treating multi-system diseases, reducing organism injury, improving exercise ability, regulating glucose and lipid metabolism, and assisting tumor treatment. An increased understanding of nitrate's impact on body homeostasis could foster a new research concept for its application in disease prevention and treatment.

1. Introduction

Homeostasis is a process of self-regulating homeostasis in which living organisms maintain the relative stability of the internal environment through self-regulating homeostasis. This process includes multiple physiological mechanisms and feedback loops involving the body's organs, tissues, and cells. Homeostasis is essential for organisms to protect them from physiological stimuli and pathogenic factors and ensure body systems' proper functioning.

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Homeostasis disorders can lead to a variety of diseases and health problems.[1] As an emerging medical concept, homeostasis medicine aims to study the role of homeostasis in health and diseases and explore the laws and regulation strategies of homeostatic balance in the body to achieve health maintenance and disease prevention and treatment.[2]

Oral health is an integral part of systemic health, closely related to the occurrence and development of various systemic diseases, the basis for maintaining health and preventing diseases, and closely associated with systemic homeostasis.[3,4] Studies[2,5,6] have found that nitrate ingested through the oral cavity is absorbed into the blood in the gastrointestinal tract and enters systemic circulation. About 25% of the nitrate in the blood is taken up and transported to the saliva through the salivary glands so that the concentration of nitrate in the saliva is about 10–20 times the level in the blood, attracting widespread attention from the scientific community. With the deepening of research, the cellular biological role of nitrate as a nitric oxide (NO) donor and mediated by the nitrate transport channel sialin has gradually been discovered, and the beneficial effect of nitrate on homeostasis has steadily been clarified. As an essential system for maintaining homeostasis, nitrate is a direction worthy of attention in homeostatic medical research.[7]

This article focuses on people's cognitive process of nitrate and the relationship between nitrate and body homeostasis, aiming to help readers understand the reasons why nitrate maintains body homeostasis and the clinical application scenarios of promising new-generation drugs to provide a reference for further research on nitrate as medications to prevent and treat diseases.

2. Overview of nitrate

Nitrate is a general term for nitric acid-derived compounds, generally a salt formed by reacting metal ions or ammonium ions with nitrate ions. Common nitrate is sodium nitrate, potassium nitrate, ammonium nitrate, calcium nitrate, lead nitrate, cerium nitrate, etc. Nitrate is easily soluble in water and is a ubiquitous nitrogen-containing compound. The primary source is nitrogen-fixing bacteria nitrogen fixation formation, or at lightning-high temperatures, nitrogen and oxygen in the air are directly synthesized into nitrogen oxides, soluble in the rain to form nitric acid, and then react with minerals on the ground to generate nitrate. Nitrate is an essential nutrient and signaling molecule for plants for growth and development.[8] Animals obtain organic nitrogen (protein) or inorganic nitrogen (nitrate) through drinking water or the food chain (Fig. 1).

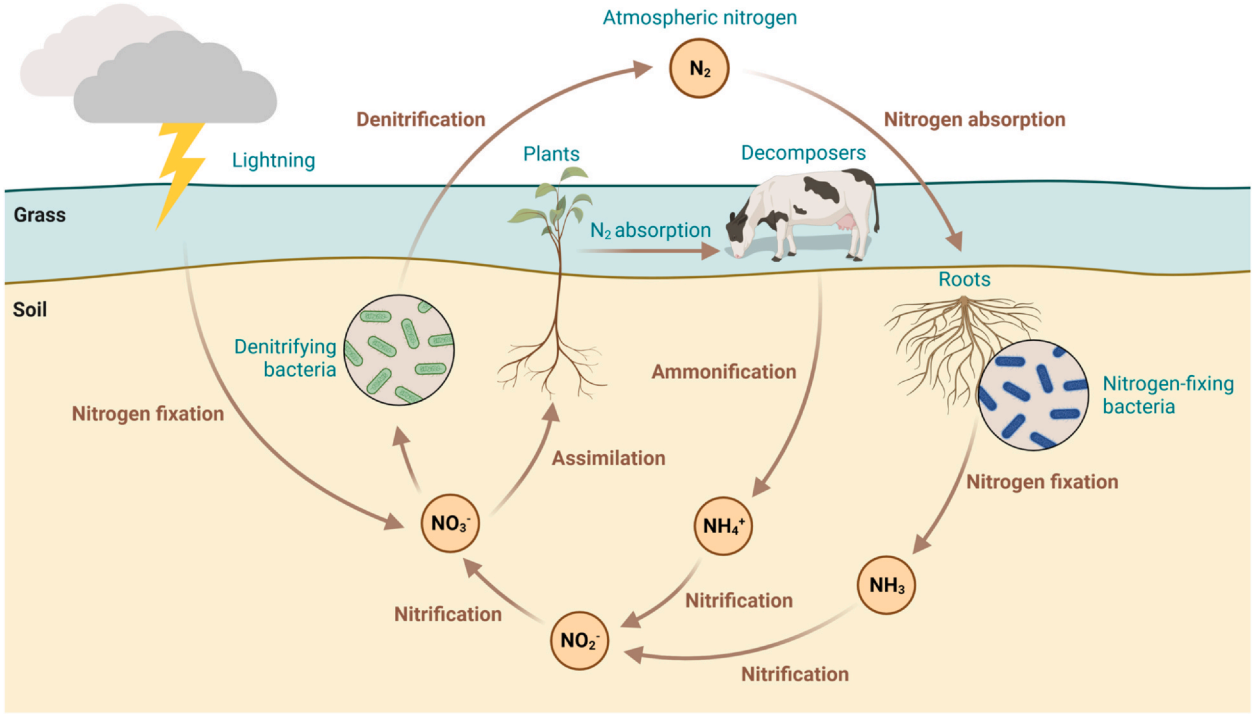

Fig. 1. Generation and circulation of nitrate in nature. N_2 and O_2 in the air generate nitrogen oxides under the action of high-energy nitrogen fixation, which are dissolved in rainwater to form NO_3^-, and then react with minerals on the ground to form nitrate. Nitrate in soil was reduced to N_2 by denitrifying bacteria and returned to the atmosphere. Subsequently, nitrogen-fixing bacteria of plants transform N_2 into NH_3 through nitrogen fixation, and NH_3 is nitrated to generate NO_2^- and NO_3^-. Plants absorb nitrogen nutrition from the soil and assimilate inorganic nitrogen into organic nitrogen such as protein, which is ingested and utilized by animals. Organic nitrogen in animal and plant residues is decomposed into NH_4^+ by microorganisms through ammoniation, and NO_2^- and NO_3^- are generated again. (Created with BioRender.com).

2.1. Medical application of nitrate

The application history of nitrate in medicine can be traced back to the ancient *Shennong Materia Medica*, which has a history of more than 2000 years. Saltpeter refers to nitrate minerals processed and refined crystals or artifacts. It is a commonly used traditional Chinese medicine in the motherland, and the main component is potassium nitrate. Ancient names include "slaked stone," "fire nitro," "flame nitro," "gold stone," and so on. Based on old medical records of saltpeter, it relieves pain, removes saprophytic muscle, dissolves phlegm and soft and firm knots, opens up the secret, relieves moisture and yellowing, warming and activating blood, etc. It can treat internal, external, gynecology, five senses, and other diseases.[9] In the West, British physician Lander Brunton first reported the antianginal effects of isoamyl nitrite in the *Lancet* in 1867.[10] It was not until 1878 that William Murrell accidentally discovered that nitroglycerin could slow the symptoms of angina and lower blood pressure, so he tried to treat patients with diluted doses of nitroglycerin and published his research in the *Lancet* in 1879. From then on, nitroglycerin began to be widely used to treat acute angina caused by coronary artery stenosis.[11]

2.2. Nitrate and cancer

Cancer is the leading cause of death worldwide, with one-third of cancers occurring due to behavioral and dietary risks.[12,13] Among them, the relationship between nitrate in the diet and cancer has been a hot topic of debate. High intake of nitrate was once considered a carcinogen. The reason for this view is the occurrence of endogenous nitrosation, leading to the formation of N-nitroso compounds (NOCs), and NOC-induced alkylation damage may cause cancer mutagenesis. However, this mutational damage has not been directly observed in the patient's tumor.[14,15] In addition, according to the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) and the European Food Safety Authority, there is currently insufficient evidence to suggest that nitrate in food and drinking water is carcinogenic.[16] Processed meat consumption is classified as "carcinogenic to humans" (Group 1) according to the IARC classification, and nitrate or nitrites ingested under conditions that will lead to endogenous nitrosation are classified as possibly carcinogenic to humans (Group 2 A).[17]

Epidemiological results have yielded mixed and complex results regarding the relationship between cancer and dietary nitrate and nitrite intake. Some studies showed positive associations, some showed negative correlations, and most studies showed no associations. In a systematic review and meta-analysis,[18] researchers assessed the relationship between dietary nitrate and nitrites and cancer risk in specific parts of humans. Forty-one articles were included in the analysis, covering 13 different cancer sites. The results showed that increased nitrite intake was associated with an increased risk of bladder and stomach cancer but a reduced risk of pancreatic cancer. Increased nitrate intake is associated with decreased kidney and bladder cancer risk. When comparing the highest and lowest intake categories, high nitrate intake was associated with an increased risk of thyroid cancer (odds ratio (OR) = 1.40, 95% confidence interval (CI): 1.02, 1.77). Combining all intake categories and comparing them with the lowest class, higher nitrite intake was associated with an increased risk of glioma (OR = 1.12, 95% CI: 1.03, 1.22). Studies on the carcinogenic risk of nitrate in drinking water show that there is no correlation between the long-term average nitrate level in public water supply and bladder cancer.[19,20] Another study noted that the risk of stomach cancer doubled for every 10 mg/L increase in nitrate, but it was not associated with colorectal, esophageal, pancreatic, brain, and non-Hodgkin lymphoma.[21] In the overall diet, several recent meta-analyses[22–24] have highlighted that an overall diet with high nitrate levels is positively associated with an increased risk of colorectal and ovarian cancer, and a comprehensive diet with moderately high or moderate nitrite is positively associated with an increased risk of gastric cancer. Regarding other cancer sites, the number of studies is very scarce, especially breast and prostate cancer. The National Institutes of Health and the Association of Retired Persons (NIH-AARP) Diet and Health Study[25] found a positive association between nitrite in processed meat and postmenopausal breast cancer risk. Still, no association was observed in the Iowa Women's Health Study.[26] In studies[27,28] on prostate cancer, a positive correlation between nitrate and nitrite intake in processed meat was similarly highlighted. Although there are many epidemiological studies on nitrate and cancer, most do not distinguish between nitrate/nitrites in natural and food additives. In addition, no studies have provided detailed information on specific nitrate/nitrite food additives. There was also a lack of reasonable adjustment for confounders in statistical analysis.[18] Antioxidants (inhibitors of NOC formation) are present in natural sources of nitrate and nitrites (mainly fruits and vegetables). They may reduce nitrosamine production of nitrate and nitrites of natural origin.[29]

In animal experiments, in a 2021 study,[30] researchers added 10 mmol/L nitrate to the drinking water of 15-month-old Wistar rats and monitored their health for the rest of their life cycle. The results showed that the incidence of colorectal cancer did not increase compared to the natural drinking water group. Another study[31] conducted in 7-week-old C57BL/6 mice came to similar conclusions. The researchers gave 7-week-old C57BL/6 mice a low-nitrate diet, added $NaNO_3$ (1 mmol/L) to drinking water at 20 weeks of age, and subsequently monitored the health of each

group. The results showed that nitrate supplementation was associated with trends towards improved insulin response, lower plasma interleukin (IL)-10, and improved survival, but not with cancer. In short, the previous view that nitrate can cause cancer has gradually changed. Although the epidemiological results are still controversial, the results of animal experiments do not suggest an increased risk of cancer.

3. Source and metabolism of nitrate

3.1. Source of nitrate

The body obtains nitrate in two main ways: exogenous intake and endogenous production. The endogenous source is the oxidation of NO. NO is synthesized by the arginine pathway and is regulated by nitric oxide synthase (NOS) and its redox state. NOS in the body is divided into constitutive NOS (cNOS) and induced NOS (iNOS). In a healthy form, the body synthesizes about 0.5–1 mmol/L endogenous nitrate daily. In the inflammatory state, due to the activation of iNOS, NO levels increase significantly, and more nitrate is oxidized into the bloodstream.[5]

The exogenous source of nitrate is mainly dietary intake, and vegetables and fruits account for more than 80% of the daily intake of nitrate and nitrite, which is the primary source of human exposure to nitrate and nitrite. Since nitrate and nitrites are widely used in food processing, they act as food additives to extend shelf life and avoid bacterial growth. The body ingests about 10%–15% of nitrate through processed meat foods,[32] and about 5% is consumed through drinking water. In recent years, nitrogenous compounds have been widely used in agricultural fertilizers to increase crop yields due to the nitrogen required for plant chlorophyll production, dramatically increasing global food production. Still, it has impacted ecosystems, especially the amount of nitrate in water and soil. Nitrogen fertilizers are a significant source of water-soluble nitrate and nitrite compounds in soil and are brought into groundwater, rivers, and drinking water through surface runoff.[33,34] The European Union[35] and the WHO[36] have stipulated that nitrate concentrations in drinking water should not exceed 50 mg/L and nitrate nitrogen concentrations should not exceed 11.3 mg/L. The guideline was formulated without specific short-term health effects (mainly methemoglobinemia and thyroid effects). China's "Sanitary Standards for Drinking Water" (GB5749-2022) stipulates that the nitrate nitrogen content in drinking water shall not exceed 10 mg/L, and this concentration is set to prevent infant nitrite poisoning. However, these safety limits are often exceeded in many countries, especially in shallow water and wells in developed and developing countries.[16,17]

3.2. Metabolism of nitrate

In terms of metabolism, the nitrate ingested by the body is rapidly absorbed by the digestive tract, and the nitrate content can be detected in the blood in 15 min, reaching a peak in half an hour, and the half-life is 5–8 h.[6] It is important to note that nitrate absorption has little first-pass effect, unlike nitroglycerin. When nitroglycerin is taken orally, it can be inactivated by about 90% due to first-pass metabolism. Most of the nitrate absorbed into the blood is excreted through urine. About 25% of the nitrate is absorbed by the salivary glands and secreted into the oral cavity. The nitrate concentration of saliva can reach 10–20 times the blood concentration and then be reduced to nitrite by symbiotic bacteria in the oral cavity, which is absorbed in the gastrointestinal tract by swallowing and produces nitrate and NO through redox reactions in blood and tissues, a process known as the nitrate-nitrite-NO pathway.[37] NO is an important signaling molecule involved in the regulation of a variety of physiological functions. Inorganic dietary nitrate is essential for human health as an additional source of NO. In addition, a nitrite may form NOC through "endogenous nitrosation." As a kind of carcinogen, NOC's carcinogenicity has been confirmed in a wide range of animal models.[38] For example, a single high-dose or short-term exposure to dimethylnitrosamine can induce Syrian hamster liver tumor,[39] rat kidney tumor,[40] and mouse lung tumor.[41] Diethylnitrosamine, as another potent carcinogen of NOC, can cause liver cancer in guinea pigs at a dose of 5 mg/kg per day,[42] and it has also been proved that it can induce precancerous cancer in hamsters, liver cancer, lung cancer, and kidney cancer in rabbits, and monkeys.[43] Besides, a high level of dibutylnitrosamine diet can cause liver cancer, esophageal cancer, and bladder cancer in mice.[44,45] MRC rats fed with N-nitrosopyrrolidine can also induce testicular tumors.[46,47] Long-term feeding of N-nitrospiperazine and N-nitrosohepatamethyleneimine imine can cause nasal cancer[48] and lung tumors in rats respectively.[49] Although NOC is carcinogenic in animal experiments, but in fact, nitrate are very stable in the body, a small part is converted to nitrite, and only a tiny amount of nitrosamines are generated under extreme conditions: (1) the overall percentage of nitrate to nitrite conversion is low, about 1%–9%;[50] (2) there is a particular time difference between nitrite formed by nitrate conversion and biogenic amines in food entering the stomach;[51] (3) the average pH value of gastric juice is about 1.8–2.0, which can rise to about 7.0 after eating, and the pH value of gastric juice within 0.5–2 h is not suitable for nitrosamine synthesis, even if the stomach recovers low pH after meals, the concentration of

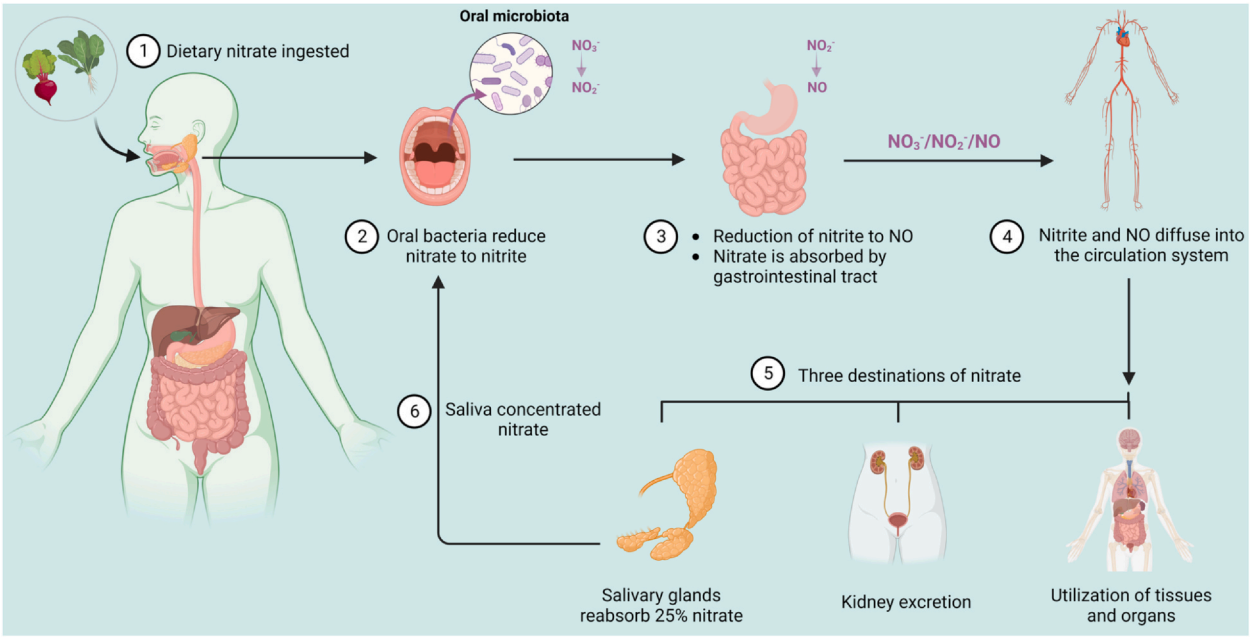

Fig. 2. Nitrate metabolism in the body. Nitrate metabolism in the body: (1) dietary nitrate ingested; (2) oral bacteria reduce nitrate to nitrite; (3) nitrate is absorbed by the gastrointestinal tract and partially reduced to NO; (4) nitrite and NO diffuse into the circulation system; (5) three destinations of nitrate: salivary gland reabsorption, kidney excretion and utilization of tissues and organs; and (6) nitrate reabsorbed by salivary glands is concentrated in saliva and discharged into the oral again. (Created with BioRender.com).

nitrite at this time does not support the further synthesis of nitrosamines;[52] and (4) during digestion, amino acid groups are protected by peptide bonds, and pepsin in the stomach only destroys tyrosine or phenylalanine peptide bonds, so there is not too much free amino acid release in the stomach, and nitrosamine synthesis substrates are limited (Fig. 2).[53]

4. Nitrate and body homeostasis

Homeostasis is a dynamic equilibrium process of self-regulation of living bodies, which maintains the relative stability of the internal environment through various physiological mechanisms and feedback loops essential for maintaining normal life activities and good physiological functions.[54] Usually, the body's systems do not exert all physiological functions, and about 50% of the physiological potential reserves are used to cope with the challenges of the external environment and adapt to the changing living environment.[55] This surplus reserve capacity allows the body to cope with overloaded environments, conducive to the rapid recovery of homeostasis. The regulatory effect of reserve function on homeostasis is reflected in all levels of the body's life activities, such as excess enzymes in organisms are the basis of metabolic homeostasis,[56] excessive sodium and potassium pumps of nerve cells are the basis of neural ion homeostasis,[57] and stem cells in the quiescent phase are the basis of tissue and organ homeostasis.[58] The role of reserve function on homeostatic regulation is one of the research focuses of homeostatic medicine, and how to mobilize and make full use of about 50% of the body's reserve function to maintain homeostasis and health is critical to reducing the occurrence of overtreatment.

Studies have found that nitrate is essential in mobilizing the body's reserve potential and maintaining homeostasis. In the past, nitrate was widely believed to be harmful to health due to concerns that nitrate could form carcinogenic N-nitrosamines. Now, new evidence suggests a paradigm shift in the role of nitrate in the diet on human health.[59] In particular, nitrate in fruits and vegetables are considered to be essential components of a healthy diet, and there is strong evidence that they are beneficial to health and can maintain a multi-level homeostatic balance in the body, including microbiota homeostasis, inflammation-immune homeostasis, and energy metabolism homeostasis, to protect the body and prevent diseases.[60] Among them, the nitrate-nitrite-NO and nitrate transport channel sialin play a key role in nitrate maintenance of homeostasis.[2,61]

4.1. Regulatory mechanism of nitrate and body homeostasis

4.1.1. Nitrate-nitrite-NO pathway

Nitrate can regulate the nitric oxide level in the body through the nitrate-nitrite-NO pathway, thereby maintaining homeostasis. NO is a gaseous signaling molecule with various critical physiological functions in the human body and plays a vital role in maintaining homeostasis. NO maintains the blood vessels' stable state by regulating vascular endothelial cells' function. It can promote the expansion of blood vessels, control blood flow and blood supply, and maintain the regular operation of tissues and organs. NO can inhibit the inflammatory response and the activation of immune cells and maintain immune homeostasis. It balances the immune response and prevents excessive inflammation and autoimmune diseases.[62] In addition, NO is involved in regulating nerve conduction and neurotransmitters, maintaining neuron homeostasis. It governs the excitability and conduction velocity of nerve cells and supports the normal function of the nervous system.[63] NO transmits signals in cells and regulates the physiological functions of cells, including cell proliferation, apoptosis, cell differentiation, and other processes, and maintains the standard structure and function of tissues and organs.[64] But NO has a short half-life, perhaps only a few milliseconds in biological tissues. Continuous NO production is essential for maintaining cell function and body health. However, the body can endogenously produce NO through NOS. NOS dysfunction occurs in advanced aging, environmental changes (hypoxia), and disease development, reducing NO synthesis and bioavailability.[65] The exogenous supplementation of the nitrate-nitrite-NO pathway can maintain the homeostasis of NO, which is also an essential mechanism for nitrate to preserve homeostasis (Fig. 3).

4.1.2. Nitrate-sialin ring-pathway

A quarter of the nitrate in the blood is actively absorbed by the salivary glands, secreted into the mouth, and finally absorbed into the bloodstream by the gastrointestinal tract, forming the intestinal saliva nitrate cycle.[66] The salivary gland plays a vital transit station in this circulation. Long-term research by Prof. Songlin Wang's group indicates that nitrate can induce specific membrane currents in salivary gland cells and doubles in an acidic environment. This

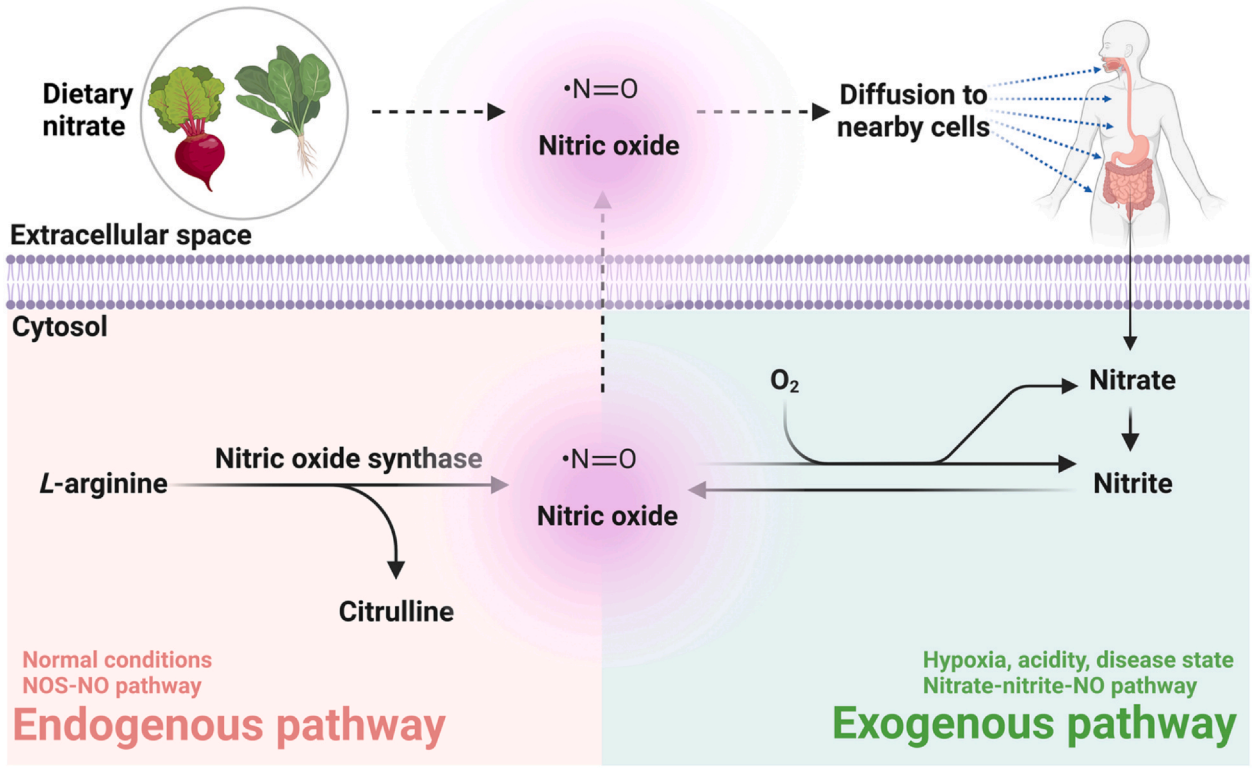

Fig. 3. Classical NOS-NO pathway and nitrate-nitrite-NO pathway. NO has many important physiological functions in the human body and plays an important role in maintaining internal balance. There are two ways for the body to acquire NO dependence: endogenous way and exogenous way. Under normal conditions, the body relies on the classical NOS-NO pathway to produce nitric oxide by endogenous *L*-arginine through nitric oxide synthase. When the body is in a state of hypoxia, acidity, and disease, the function of nitric oxide synthase is impaired, and the body supplements nitric oxide through nitrate-nitrite-NO exogenous pathway. (Created with BioRender.com).

suggests that nitrate enters the cell synergistically through the membrane and protons. After genetic testing and bioinformatics analysis, sialin was finally determined as a nitrate transport channel of mammalian cell membranes.[67] It was confirmed by sialin gene silencing cell experiments and *in vivo* experiments on viral transfection of miniature pig parotid gland that downregulation of sialin expression reduced nitrate transport capacity. Sialin is a member of the SLC17 family, SLC17A5, which is expressed in almost all tissues, with the parotid gland being the most abundantly expressed, followed by the brain, kidney, liver, pancreas, thyroid, and mandibular glands. Sialin is located in the basolateral membrane of serous acinar cells, which is also consistent with its function of nitrate transport.[6] Findings of sialin are essential for the nitrate-nitrite-NO cycle because the uptake and excretion of nitrate by the salivary glands is the first step in converting nitrate to nitrite in the oral cavity. In addition, there is a positive feedback loop pathway between nitric acid and sialin. Adding nitrate during *in vitro* cell culture can increase the expression of sialin.

In ovarian-depleted xerostomia rats, inorganic nitrate can increase the expression of sialin in the salivary glands and increase the amount of saliva secretion, alleviating fibrosis and hypofunction of the salivary glands.[68] This phenomenon also occurred in a model of radiation injury to the salivary glands in miniature pigs.[69] In addition, mutations in human *SLC17A5* genes can cause serious health problems, Salla disease, which is characterized by abnormal deposition of sialic acid, clinical symptoms such as mental retardation, nystagmus, hypotonia, and speech and cognitive impairment, and mice with total knocking of sialin die of developmental disabilities after birth.[70] Therefore, we speculate that sialin's role may be more than just a transit channel. In further research, the research group explored the cell localization of sialin and found that sialin not only exists in the cell membrane but also in the cytoplasm, mitochondria, lysosomes, and other organelle membranes. It can mediate a series of cell biological functions, including improving mitochondrial function and intracellular autophagy function. Therefore, nitrate sialin ring-mediated cell biology and nitrate regulation of NO homeostasis are important directions for future research on nitric acid-mediated homeostasis regulation (Fig. 4).

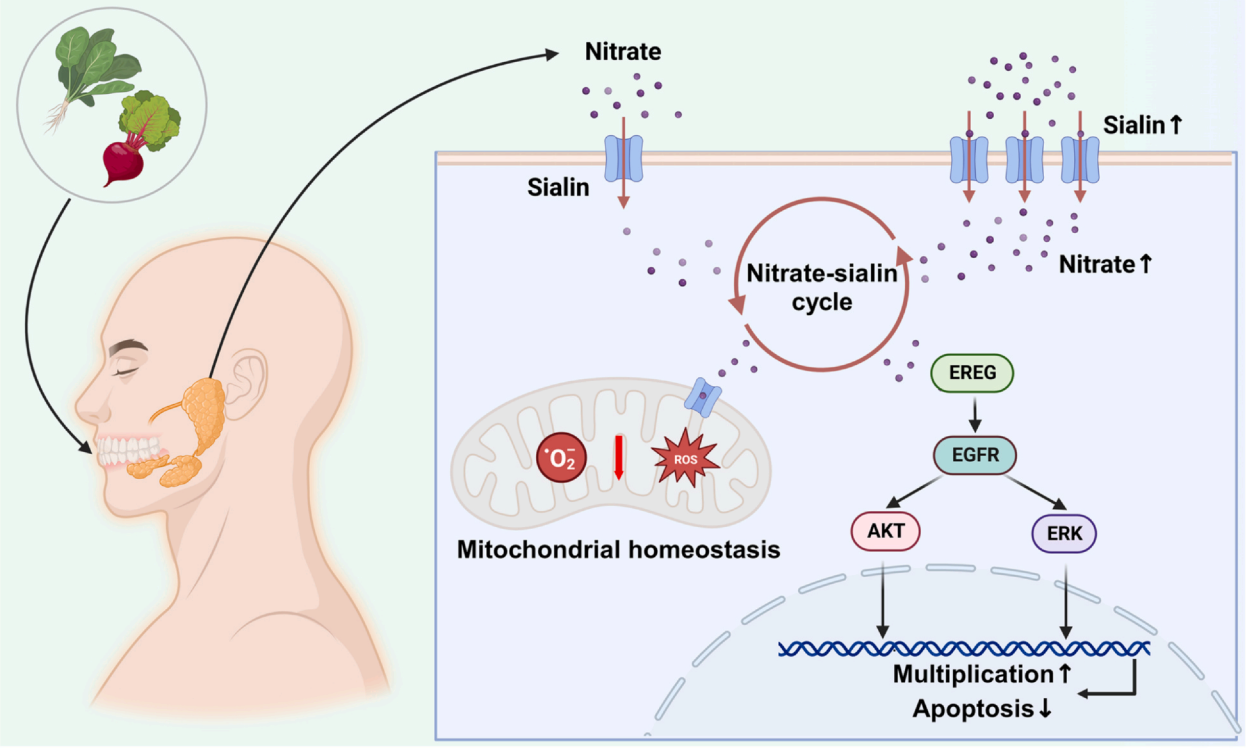

Fig. 4. Schematic diagram of nitrate-sialin feedback loop. Sialin and nitrate form a feedback loop. Sialin can transport nitrate into cells, and the increase of nitrate concentration can increase the expression of Sialin in cells, thus further transporting more nitrate into cells to play a role, such as activating EREG-EGFR-AKT/ERK signaling pathway and regulating cell proliferation and apoptosis. As well as reducing oxidative stress and improving mitochondrial function. (Created with BioRender.com).

4.2. Nitrate-mediated regulation of homeostasis

4.2.1. Bacterial homeostasis

The homeostasis of the microbiota is essential for maintaining the organism's health, and the interaction and balance of the microbiota is the key to host health. Various endogenous and exogenous factors can lead to harmful changes in the composition and metabolism of the microbiota, disrupt the balance of the microbiota, lead to microbiota dysbiosis, and cause a series of diseases.[71,72] The relationship between nitrate and flora is complex and diverse, and bacteria can use nitrate and nitrite as electron acceptors for respiration to help bacteria survive. But nitrate can also inhibit bacterial growth in various ways (Fig. 5).[73,74]

4.2.2. Inflammation-immune homeostasis

Inflammation is an integral part of the body's self-protection mechanism, which plays a crucial role in pathogen recognition and killing and the repair and regeneration of damaged tissues. The activation of immune cells often accompanies inflammation, and the vascular system is crucial in balancing inflammation and immunity.[75] During inflammation, vascular endothelial cells express chemokines to recruit and guide circulating leukocytes to inflamed tissue sites. Second, inflammation is associated with oxidative stress, which activates pro-inflammatory signals, which induces and amplifies oxidative stress through immune cells, which is beneficial for normal immune function. Under normal physiological conditions, the body initiates a self-regulation mechanism to restore homeostasis once the inflammatory stimulus is eliminated.[76]

Conversely, if the antioxidant defense system is inadequate and sustained exposure to inflammatory stimuli, excessive amounts of active substances are produced, resulting in chronic inflammation centered on oxidative stress, leading to molecular, cellular, and tissue damage. Eventually, unresolved inflammation can destroy tissue, fibrosis, and necrosis, causing various diseases.[77] In addition, different immune cell subsets involved in innate and adaptive immune responses play a crucial role in initiating and promoting disease progression, particularly macrophage phenotypic switching and neutrophil-involved signaling.[78]

More and more studies have shown that inorganic nitrate plays an essential role in the maintenance of inflammation-immune homeostasis, are potential strands to regulate inflammation-immune-vasculature interactions, can reduce oxidative stress in various diseases, reduce pro-inflammatory processes and organ damage, and improve immune cell and vascular function.[79] The mechanism depends on the enhancement of the nitrate-nitrite-NO pathway and the regulation of the nitrate-sialin ring.[80] NO can directly inhibit the activation of neutrophils and monocytes and upregulate dependent anti-inflammatory pathways such as IL-10 to reduce leukocyte recruitment, migration, and adhesion.[81] Second, inorganic nitrate may attenuate nicotinamide adenine dinucleotide phosphate (NADPH)-derived superoxide production in activated macrophages through NO-dependent mechanisms.[82] At the same time, NO can also act as an antioxidant to scavenge other, more reactive free radicals. In addition, the nitrate-sialin loop can also regulate the Ctsl-signaling pathway, thereby regulating bone marrow-derived macrophages to play an immunomodulatory role (unpublished data of Beijing Laboratory of Oral Health) (Fig. 6).

4.2.3. Energy metabolism homeostasis

Energy metabolism is an integral part of the human life process, which refers to the body's absorption, conversion, and utilization of external energy. It is closely related to the body's energy balance, weight control, disease prevention, etc. Studies have found that mice lacking a nitrate diet for a long time will have a series of metabolic abnormalities, including dyslipidemia, increased visceral obesity, insulin resistance, and impaired glucose tolerance.[83] Conversely, nitrate supplementation in various metabolic disease models and healthy volunteers demonstrated beneficial effects such as enhanced exercise capacity, induced fat browning, and improved islet function.[83] Possible mechanisms are associated with nitrate-targeting mitochondrial homeostasis, insulin release, fat browning, and oxidative stress[60] (Fig. 7).

5. Nitrate-mediated health maintenance and disease prevention

5.1. Oral diseases

The oral cavity is the second most diverse microbial community in the human body,[84] which contains all kinds of bacteria with nitrate reductase activity, among which *Veillonella*, *Actinomyces*, and *Rothia* are the most common. In contrast, others such as *Neisseria*, *Streptococcus*, *Capnocytophanga*, *Selenomonas*, *Corynebacterium*, *Haemophilus*, *Paraburkholderia*, *Fuso Bacterium*, *Propionibacterium*, *Prevotella*, and *Eikenella* have also been reported to have nitrate reducing ability in recent years.[6,85] These bacteria can denitrify nitrate into final nitrogen (N_2) by forming NO and

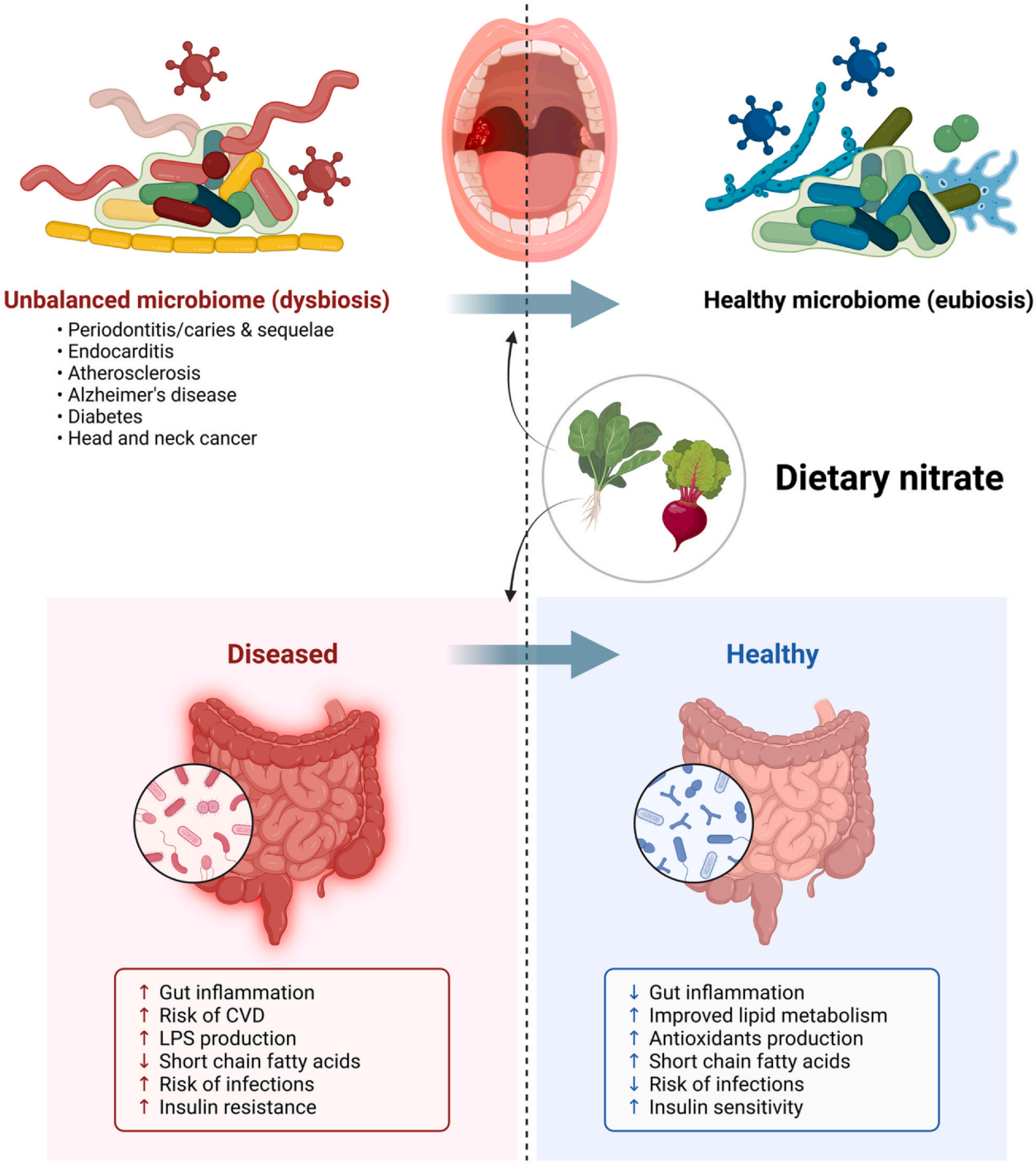

Fig. 5. Nitrate maintains a microecological balance of the oral cavity and gastrointestinal tract. Nitrate regulates the disordered and unhealthy oral and intestinal flora to a balanced and healthy state and reverses many diseases caused by it. (Created with BioRender.com).

nitrous oxide (N_2O).[86] Nitrate can prevent or reverse the imbalance of oral flora in many ways and restore plaque biofilm to a healthy state. In the process of nitrate metabolic reduction, protons, and lactic acid as carbonic acid and electron donors will be consumed, and ammonium and ammonia (a weak base) will be produced, limiting acidification.[87] In addition, the antibacterial effect of nitric oxide can reduce the level of dental caries-related bacteria such as *Streptococcus*, *Vibrio*, and actinomycetes.[88,89]

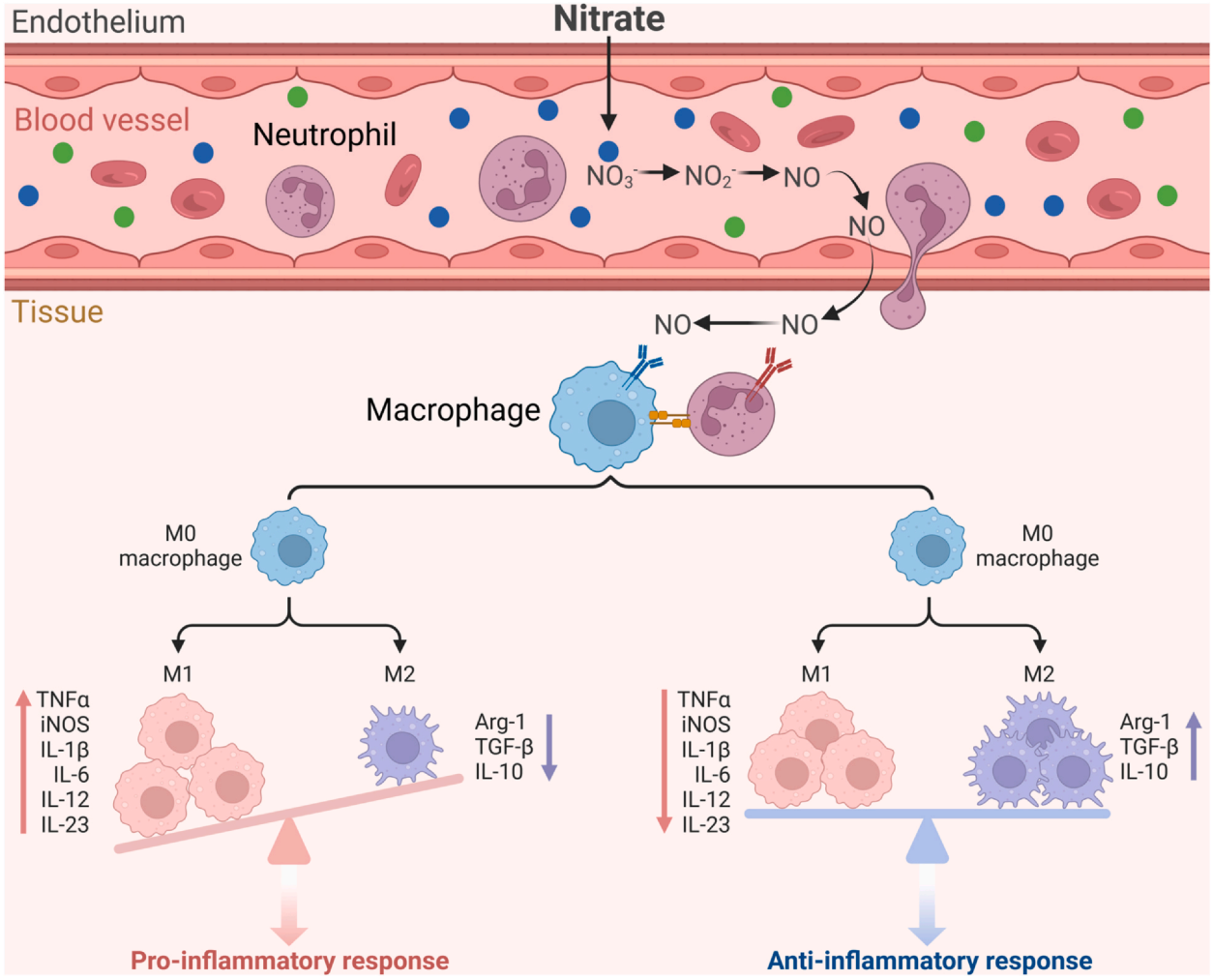

Fig. 6. Nitrate-mediated inflammatory-immune homeostasis. Nitrate-nitrite-NO can maintain the homeostasis of macrophage M1/M2, regulate the levels of various inflammatory factors, and maintain the homeostasis of inflammation-immunity. (Created with BioRender.com).

Interestingly, the pH value of the nitric oxide reduction reaction is about 5, which can adjust the occurrence of acidification by negative feedback and prevent the overgrowth of acidophilic bacteria. Clinical evidence shows that eating beetroot juice for two weeks can increase the pH value of saliva from 7.0 to 7.5. Taking nitrate before eating sugary drinks or gargling with sugary preparations can reduce the acidification of the oral environment after several hours.[90] It was also found that the number of lactic acid and fermentation bacteria decreased, the content of nitrite and ammonium increased, and the pH value of the culture system also increased in the presence of nitrate. There was no noticeable decrease within one week.[64] In addition to inhibiting the progress of dental caries, nitrate also shows the potential to resist gingivitis, periodontitis, and halitosis. Nitrate can reduce the number of periodontal pathogens, including *Fusobacterium*, *Sclerotinia*, *Bacteroides*, *Prevost*, and *Porphyromonas* levels in saliva. Supplementing lettuce juice rich in nitrate for two weeks can significantly change plaque composition and reduce gingival inflammation.[91] This effect may be related to the inhibition of oxidative stress-sensitive pathogens by nitric oxide, which, as a signal of biofilm diffusion, can effectively limit the accumulation of plaques. In addition, nitrate may benefit halitosis patients by eliminating halitosis-related pathogens, inhibiting the metabolic pathway of volatile sulfur compound (VSC) production, and stimulating the utilization of hydrogen sulfide (H_2S).[64]

5.2. Diseases of the digestive system

Dietary nitrate enters the circulatory system through digestion and absorption in the gastrointestinal tract, so the gastrointestinal tract is the direct target organ of nitrate action. The stress models of “bungee jumping” and “bound

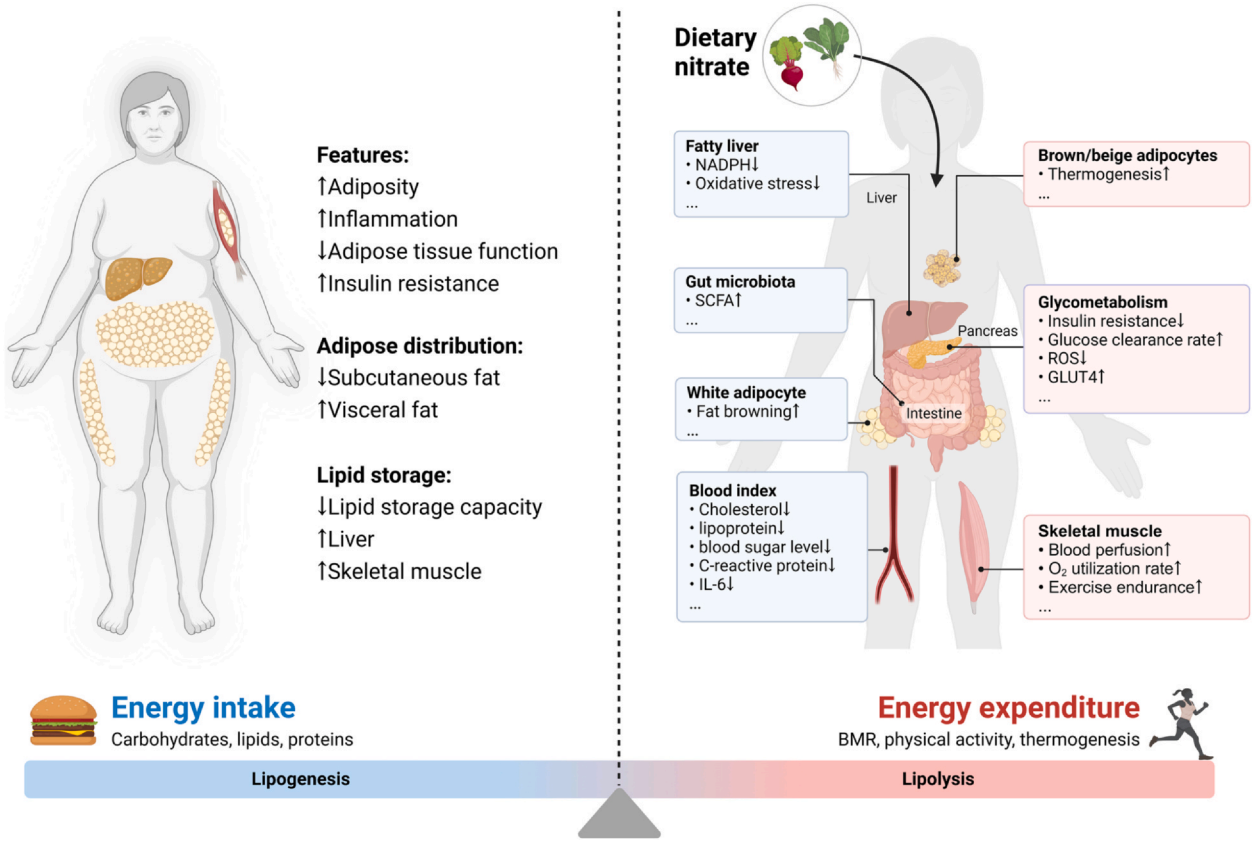

Fig. 7. Nitrate-mediated Energy metabolism homeostasis. Nitrate participates in the regulation of energy metabolism homeostasis by targeting mitochondrial homeostasis, insulin release, fat browning, and oxidative stress, and plays a role in enhancing exercise capacity, inducing fat browning and improving insulin function, and reversing diseases including dyslipidemia, visceral obesity, insulin resistance, and impaired glucose tolerance. (Created with BioRender.com).

immersion" confirmed that under stress, the salivary glands actively transport and secrete nitrate to form high-concentration saliva nitrate, which is swallowed into the gastrointestinal tract, and through the nitrate-nitrite-NO pathway, the gastrointestinal blood flow is increased, the gastric mucus layer is thickened, and the gastrointestinal ulcer, erosion, bleeding, and perforation are reduced, to protect the gastric mucosa from stress injury.[92,93] In addition, nitrate participates in regulating the homeostasis of gastrointestinal flora. Dietary nitrate has been reported to save gastrointestinal microbiota imbalance during antibiotic treatment. Long-term nitrate supplementation in rodents can effectively prevent metabolic syndrome caused by intestinal microbial imbalance.[92,94] In mechanism, nitrate is reduced to nitrite by oral bacteria. Then NO is generated under gastric acid's catalysis, which enhances gastric juice's antibacterial effect and inhibits the number of *Escherichia coli* and *Candida albicans*. Inflammation in the intestine of patients with inflammatory bowel disease can also lead to the production of NO, which is very powerful in inhibiting bacteria.[95] In addition, nitrate can provide nitrogen nutrition for bacteria and be denitrified. It is unclear whether dietary nitrate can stimulate the transformation of the intestinal microbial community.[96] However, there is some evidence that nitrate can prevent and treat diseases by improving the abundance of beneficial bacteria. For example, in the obese mouse model induced by a high-fat diet, nitrate can increase the mass of *Bacteroides* and *Alistipes* in the intestine, rebalance the intestinal microflora, and reduce the occurrence of obesity.[97] In dextran sulfate sodium (DSS)-induced chemical inflammatory bowel disease, nitrate pretreatment can also increase the abundance of lactobacillus, lactobacillus, and rumen coccidae, reduce the inflammatory index of the colon, and improve the length of the colon.[98] In addition, oral nitrate can prevent the development of nonalcoholic fatty liver disease in mice, and the mechanism is mainly through the regulation of the Ctsl-Nrf2 pathway by sialin protein, thus regulating the immune regulation of bone marrow-derived macrophages in the liver (unpublished data of Beijing Laboratory of Oral Health).

5.3. Diseases of the cardiovascular system

There is solid evidence that dietary interventions to modulate inflammatory processes are key lifestyle strategies for maintaining cardiovascular health and preventing cardiovascular diseases such as atherosclerosis and hypertension.[99] In addition, NO plays a crucial role in cardiovascular homeostasis. The decline in the production and utilization of NO is closely related to cardiovascular disease risk factors such as vascular aging and endothelial dysfunction.[100] Therefore, inorganic dietary nitrate has attracted attention as an additional source of NO.[101] As early as the eighth century in ancient China, a manuscript from the Mogao Caves documented nitrate's use in treating acute cardiovascular diseases. Organic nitrate drugs have been widely used in treating cardiovascular diseases, and their effect mainly depends on NO production. However, prolonged use can cause adverse effects such as drug resistance, endothelial dysfunction, syncope, or orthostatic hypotension.[102,103] Inorganic nitrate, also a source of NO supplementation, has not been identified with these problems. Inorganic dietary nitrate produces NO via the intestinal saliva nitrate-nitrite-NO pathway. Unlike NOS-resistant NO production, nitrite reduction to NO in this reaction is independent of oxygen. NO production via the nitrate-nitrite-NO pathway accelerates even under hypoxic or acidic conditions, which is critical in patients with chronic cardiovascular disease.[37] Therefore, inorganic nitrate has excellent potential in preventing and treating cardiovascular diseases. The antihypertensive effects of inorganic nitrate have been observed in rats with metabolic syndrome in multiple animal models, including single nephrectomy,[104] a high-salt diet,[104] and high-fructose-induced metabolic syndrome.[105] In a meta-analysis, including healthy subjects and hypertensive patients, inorganic nitrate ingestion resulted in a 4.80 mmHg (1 mmHg = 0.133 kPa) reduction in resting systolic blood pressure (SBP) and a 1.74 mmHg reduction in resting diastolic blood pressure (DBP).[106] Another study suggested that inorganic nitrate has a lower threshold and longer duration of action against hypertension and appears to be superior to the presence of organic nitrides.[107] In addition, inorganic nitrate has beneficial effects in improving the quality of life of patients with heart failure. In mice with cardiac insufficiency[108] and rat models of heart failure,[109] nitrate intervention enhanced left ventricular systolic diastole function, increased ejection fraction, and gained more pronounced during exercise. The same effect was shown in human trials, where nitrate-rich beetroot juice concentrate increased maximum muscle speed, strength, and endurance during exercise in patients with heart failure[110] or older people with reduced cardiac function.[111] Of note, inorganic nitrate appear to inhibit platelet activity and reduce platelet-leukocyte aggregation adhesion by producing NO, which helps reduce the occurrence of blood clots and may be a promising candidate for preventing postoperative pulmonary embolism.[112] Data from another animal study showed that inorganic nitrate supplementation could reduce macrophage content and increase smooth muscle content in atherosclerosis-susceptible mouse plaques, resulting in a more stable plaque phenotype, which is practical in preventing the development of atherosclerosis.[113,114]

5.4. Iatrogenic injuries

Nitrate have shown therapeutic potential in reducing iatrogenic injuries, including radiation and drug-induced injuries. As a standard adjuvant treatment for cancer, radiotherapy's side effects seriously affect patients' quality of life.[115] Animal experiments have shown that inorganic nitrate is effective in preventing radiation damage. In a mouse model of whole-body gamma irradiation, nitrate pretreatment can restore the levels of peripheral red blood cells, white blood cells, and platelets one week after radiotherapy, reducing radiotherapy-induced weight loss.[116] In addition, hypo salivary gland function caused by radiotherapy for head and neck tumors can lead to dry mouth, mouth ulcers, dental caries, periodontal disease, infectious oral mucosa, or sialadenitis, and even affect the occurrence and development of systemic diseases. There is no effective clinical management method, and nitrate may provide a new treatment for solving ischemia-reperfusion (IR)-induced hypo salivary gland function. In the IR-induced model of micro porcine salivary gland hypofunction, nitrate alleviated IR-induced parotid gland injury in a dose-resistant manner, promoted the uptake and utilization of nitrate by parotid gland tissue by increasing the expression of sialin in parotid gland tissue, and the nitrate-sialin feedback loop activated the EGFR-AKT-MAPK signaling pathway, enhanced parotid gland cell proliferation and inhibited apoptosis.[69] In another IR-induced mouse salivary gland injury experiment, inorganic nitrate also showed potential as a salivary gland protector. Pretreatment of 2 mmol/L nitrate successfully alleviated the decrease in saliva flow rate and weight loss in mice, and further studies found that this protective effect was achieved by reducing NLPR3-mediated cell pyrosis and maintaining mitochondrial homeostasis.[117] In addition, nitrate reduces gastrointestinal damage and ulceration caused by nonsteroidal antiinflammatory drugs by increasing blood flow and mucus thickness in the gastric mucosa.[118]

5.5. Postoperative complications

Postoperative complications are one of the leading causes of treatment failure and poor prognosis, and nitrate shows considerable potential in alleviating postoperative complications. Ovariectomy often causes complications such as osteoporosis and decreased saliva production. Animal studies have shown that nitrate treatment can improve bone loss in ovariectomized (OVX) models, increase saliva flow rate, and reduce submandibular gland atrophy and fibrosis.[119] In addition, IR injury (IRI) is common in various interventional surgeries, organ transplantation operations, and flap-free surgery, which refers to the restoration of blood flow perfusion within a certain period after the interruption of blood supply to tissues and organs in a short period, which not only cannot restore the function and structure to normal but will aggravate the injury. Among them, inflammation and oxidative stress are the leading causes of IRI.[120] The protective effects of nitrate and nitrites on IRI damage have been observed in various animal models. For example, nitrate pretreatment can preserve the glomerular filtration rate and renal blood flow in mouse models of renal IRI[121] and alleviate hepatic IRI damage in mice by activating the Nrf2 pathway and regulating oxidative stress.[122] In addition, dietary nitrate helped tissue edema of rat-grafted flaps by controlling oxidative stress and reducing the inflammatory response, increasing the survival rate of flaps.[123] Another study on inorganic nitrate for postoperative IRI after coronary artery bypass grafting found that patients treated with inorganic nitrate had 18% less perioperative bleeding than controls.[124]

5.6. Improvement of exercise capacity

Dietary nitrate has attracted increasing attention as a sports nutrition supplement. Since 2007, the first research on nitrate supplementation could improve exercise efficiency was published, a wave of nitrate-sports medicine has gradually been set off.[125] A meta-analysis[126] of data from 1705 participants, including 123 studies, showed that nitrate intake did improve the ability to perform in exercise. NO is known to be essential in vascular and metabolic control. An increase in NO can affect mitochondrial respiration and biological activity, increase blood flow to active muscles, and reduce the depletion of adenosine triphosphate (ATP) during muscle contraction. With nitrate as an NO supplement, the gain of human exercise capacity is multifaceted.[127]

First, cardiovascular nitrate improvements can help improve exercise capacity, such as increasing muscle blood perfusion and accelerating nutrient delivery and waste excretion. In addition, exercise is usually accompanied by a decrease in muscle oxygen, and the conversion rate of oxygen determines the strength of exercise performance. In a double-anonymized, placebo-controlled study, dietary nitrate reduced oxygen consumption during physical activity in healthy young subjects, but interestingly, there was no difference in lactic acid production. These results suggest that supplementation with nitrate does not increase energy production but makes aerobic work more efficient. The team then examined the effect of resting metabolic rate in healthy subjects and found that nitrate reduced oxygen consumption by about 4% at the same exercise intensity.[128] Human exercise physiology holds that the oxygen cost for a submaximal exercise is essentially fixed at a given operating rate, regardless of age and health. However, nitrate achieves greater operating output at the exact energy cost, significantly improving muscle efficiency.

In addition, nitrate has been observed to be beneficial in delaying fatigue and improving exercise tolerance. In a 50-mile cycling endurance race, dietary nitrate supplementation improved an athlete's performance by 0.8%. Drinking nitrate-rich beetroot beverages for six consecutive days can improve a soccer player's ability to engage in high-intensity interval activities and significantly reduce fatigue due to the intensity and frequency of sprints.[129] Similarly, the same beneficial effects were observed in both running[130] and rowing.[127] In addition, muscle oxidation function and exercise tolerance are reduced in hypoxic environments, such as high altitude or underwater exercise. The nitrate-nitrite-NO pathway is not affected by low pH and hypoxia, suggesting the potential of nitrate to improve hypoxic training. In a high-intensity knee extension exercise performed in a low-oxygen environment, nitrate supplementation was found to restore the metabolic balance of muscles and improve exercise tolerance to normoxic levels. In addition, the evaluation of the muscle oxygenation index provides some evidence. In hypoxic cyclic exercise experiments, they can increase the total oxygenation index of the lateral femoral muscle and gastrocnemius muscle (approximately 4%).[131]

Studies have found that nitrate concentration in skeletal muscle is much higher than in the blood, and nitrate in muscle is extremely sensitive to dietary nitrate supplementation and deficiency. At the same time, nitrate gradients in the muscle-blood-liver suggest that our skeletal muscle may be an endogenous nitrate reservoir in the body. Animals on a 7-d low-nitrate diet had muscle nitrate levels that were nearly depleted (nitrate starvation), while reintroducing a nitrate-rich diet returned to normal levels in less than three days, suggesting that nitrate is essential for muscle metabolic homeostasis.[61]

5.7. Disorders of glycolipid metabolism

Obesity is the eighth leading cause of death and disability globally and already causes a considerable health burden worldwide. The number of overweight/obese people worldwide will exceed 4 billion in 2035, of which nearly 2 billion will be obese. The obesity rate among adult men will increase from 14% in 2020 to 23% in 2035; The obesity rate among adult women will increase from 18% to 27%. Obesity is not only a weight problem but also a cause of many diseases, such as diabetes, high blood pressure, cardiovascular disease, and cancer.[132] In adipose tissue, the uptake and storage of fatty acids are essential in maintaining lipid balance in the body. Fatty acids in adipose tissue are taken up by fat cells and stored mainly in white adipose tissue (WAT). Under starvation conditions, triglycerides in white adipose tissue are hydrolyzed, and the fatty acids and glycerol released from this are transported back to the liver, where gluconeogenesis and ketogenesis occur to replenish the source of energy in the blood as needed.[133] However, in adipose tissues of obese patients, the expression of critical genes that mediate lipolysis, lipid production, and lipid storage decreases, but the expression of genes related to the proliferative growth of adipose tissue increases. This eventually leads to dysfunction of fatty tissue, the development of disorders of glycolipid metabolism, and an increased risk of diabetes.[134,135] Obesity has been reported to be a significant risk factor for diabetes, leading to insulin resistance that usually precedes diabetes.[136] In addition to the increase in body fat, obese people often have the redistribution of body fat, that is, the increase in visceral fat and ectopic deposition of fat, such as liver, muscle, and other parts, causing health problems such as fatty liver or muscle weakness.[137] Therefore, maintaining the homeostasis of the body's glucose and lipid metabolism is essential to protect the body's health.

Studies have found that oxidative stress and reduced NO bioavailability are essential to obesity and diabetes. Oxidative stress causes metabolic disorders through various mechanisms, one of which is to reduce the bioavailability of NO by directly removing NO or decoupling NOS. As obesity is a hypoxia state with low blood flow, the production of endogenous NO itself is inhibited.[138,139] As mentioned earlier, nitrate has the effect of reducing oxidative stress and compensating for NO loss. Nitrate are, therefore, promising for the management of obesity and diabetes.[140] In obesity, the metabolic activity of fat cells increases, and a large amount of protein synthesis is required. When the endoplasmic reticulum cannot meet protein synthesis needs, it will cause endoplasmic reticulum stress due to improper folding. At the same time, obesity also leads to instability in mitochondrial metabolism, leading to oxidative stress through various biochemical processes. Among them, NADPH oxidase-mediated superoxide production is the primary source of reactive oxygen species (ROS).[141,142] In the prevention and treatment of obesity, a systematic review and meta-analysis of 11 animal sample sizes of 395 animals showed that the final weight of the nitrate supplementation intervention group was significantly lower than that of the control group (95% CI: 6.24–27.38, $P = 0.002$) and higher than 72.94 mg nitrate doses of L^{-1} d^{-1} and nitrate exposure over eight weeks resulted in more significant weight differences.[143] In addition, nitrate attenuated NADPH oxidase activity, reduced ROS production, and improved NO bioavailability in cardiometabolic models. This is essential for preventing fatty liver disease, as increased NADPH oxidase activity in the liver correlates with the degree of lipid accumulation. In a diet-induced animal model of fatty liver, dietary nitrate can attenuate the development of stratification by inhibiting NADPH oxidase activity.[144] In addition, nitrate can also promote the conversion of white fat to comprehensive adipose tissue, referred to as fat browning. Brown fat is characterized by increased energy expenditure and heat production, and studies have found that nitrate can increase the expression of thermogenic genes in brown fat, as well as induce the expression of brown fat cell-specific genes and proteins in white fat to promote browning, increase oxygen consumption and fatty acid oxidation of fat cells. Interestingly, this gain is more pronounced in hypoxic conditions.[145]

In preventing and treating diabetes, acute nitrate intake can improve glucose clearance and insulin resistance in adenosine A2B-deficient mice.[146] In the type 2 diabetes (T2D) rat model induced by injection of streptozocin and niacinamide, nitrate alone significantly reduced plasma glucose and increased pancreatic superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity, GPx and SOD synergistically cleared multiple ROS, reduced oxidative stress damage of islet cells, and restored insulin secretion function.[147] In addition, aging is associated with an increased risk of T2D, which leads to decreased function of pancreatic islets β cells with age, resulting in reduced insulin secretion and weakened ability to regulate glucose, leading to diabetes. The study found that dietary nitrate supplementation for two weeks improved glucose clearance in older rats, while there was no improvement in younger rats.[130] These beneficial effects have been reported to be associated with increased expression of GLUT4, improved insulin response, decreased gluconeogenesis, and inflammation in insulin-sensitive tissues.[148–150]

Obesity and diabetes are closely related and often develop at the same time. Some animal studies have found that mice lacking eNOS develop disorders of glycolipid metabolism with age. Still, nitrate intervention can reduce blood lipid and glycosylated hemoglobin levels, reduce fat accumulation, and improve glucose tolerance.[151] Other studies have observed decreased nitrate and nitrite levels in plasma in rat models with oophorectomy, gradually developing visceral obesity and insulin resistance. However, dietary nitrate supplementation restores blood nitrate and nitrite levels, causes weight loss, improves insulin resistance, and lowers blood sugar. These results support the potential

therapeutic role of nitrate in postmenopausal women with features of metabolic syndrome.[152] In addition, in mouse models of high-fat and high-fructose diets, nitrate interventions reduced cholesterol, lipoprotein, blood glucose, and inflammatory factor levels.[153] Nitrate is essential for glycolipid metabolism homeostasis and preventing obesity and diabetes. However, evidence from human trials is still lacking for these results.

5.8. Cancer prevention and treatment

The occurrence and development of tumors are closely related to the homeostasis imbalance. At different stages of development, the direction of tumor homeostasis imbalance is other. Taking pro-oxidation and antioxidants as an example, early in cancer, appropriate concentrations of reactive oxygen species can stimulate tumor formation and support its development, and the application of antioxidants can help inhibit tumors. In the later stage of tumors, to cope with the oxidative stress death of tumor cells caused by excessive concentration of reactive oxygen species, the oxidative stress capacity of tumor tissues is enhanced, and pro-oxidants can destroy the redox homeostasis of tumor tissues and kill tumor cells.[154,155] The metabolic process of nitrate in the body is filled with various redox reactions, which have a regulatory effect on the redox homeostasis of cancer. As a NO donor, synthetic nitrate can provide sustained high concentration of NO, improve NO homeostasis in tumor tissues, reduce the expression of early stress response genes, and enhance the therapeutic effect of tumor.[156] For example, 0.1 nmol/L nitroglycerin combined with azithromycin can reduce the growth of human prostate cancer xenografts in mice.[157] Another study also found that nitroglycerin may inhibit the expression of a disintegrin and metalloproteinase domain 10 (ADAM10) and subsequent MHC class I-related molecule A (MICA) shedding by inhibiting the accumulation of hypoxia-inducible factor (HIF)-1α in tumor cells, thus weakening the growth of human prostate tumors in nude mice by relying on innate immune effector cells.[158] In addition, sodium nitroprusside can induce apoptosis of cutaneous T-cell lymphoma.[159] Nitrate increases the chemosensitivity of oral squamous cell carcinoma to cisplatin through REDD1/AKT signaling pathway,[160] and another nitric oxide donor, DETA/NO, has also been found to enhance cisplatin-mediated cytotoxicity and reduce the tumor volume of xenotransplantation melanoma.[161]

In addition, the nitrate-nitrite-peroxynitrite ($ONOO^-$) system may kill tumor cells in specific environments. Nitric oxide reacts with neutrophil-derived superoxide *in vivo* to produce the potent oxidant $ONOO^-$. $ONOO^-$ plays vital roles in biological processes, such as regulating mitochondrial function inducing apoptosis and necrosis.[162,163] In addition, $ONOO^-$ can directly cause DNA damage in cells, inhibit the expression of DNA repair enzyme poly(ADP-ribose) polymerase (PARP), and ultimately increase DNA damage caused by radiotherapy and inhibit tumor growth.[164] Tumor-specific ONOO nanogenerators can improve drug delivery and enhance the effect of tumor chemotherapy.[165] But nitrate-nitrite-$ONOO^-$ is high pH and low oxygen environmental dependent. Below pH 7, > 90% of free peroxynitrite is isomerized to nitrate.[137] There are few studies on the nitrate-nitrite-$ONOO^-$ pathway, and its anti-cancer effect needs to be explored. In summary, the role of nitrate in tumor redox homeostasis is complex, and these effects may impact the growth and development of tumor cells. However, further research needs to determine the specific mechanism of action and the extent of the impact (Fig. 8).

6. Safety of nitrate

Although nitrate has many health benefits, it should be noted that the safety of nitrate is also related to individual differences, intake routes, and intake doses.[59,166] The sources of dietary nitrate intake include plant sources, animal sources, and drinking water. Among them, plant sources consume a lot of nitrate, which is generally considered unlikely to have health risks. However, there are concerns about excessive nitrate and nitrite intake from processed meat. Although these compounds are not carcinogenic, they may react with other compounds in food during cooking or the digestive tract, which may form the carcinogenic substance NOC.[167] Especially red meat, a rich heme iron source, is a catalyst for the human body to synthesize endogenous NOC. The NOC level of men who eat a lot of red meat every day is significantly higher than that of men who eat little red meat.[168,169] However, this relationship can be distorted or changed by nitrosation inhibitors such as various polyphenols and dietary ingredients such as vitamins E and C in fruits and vegetables.[170] In addition, drinking water with excessive nitrate seems to bring health risks. In 1940, blue baby syndrome caused by healthy water with high nitrate concentration was first reported, also known as methemoglobinemia in infants.[171] This is mainly related to the production of nitrite. Excessive nitrite can oxidize normal hemoglobin, making it lose its oxygen-carrying ability, leading to tissue hypoxia and adverse reactions such as unconsciousness, arrhythmia, coma, and even shock.[33,172] However, nitrate is relatively safe in moderate intake. According to the assessment of the European Food Safety Agency, the daily allowable intake (ADIs) of nitrate is 3.7 mg/kg body weight, and the content of each nitrate as a food additive should not exceed 5% of the total nitrate content in food.[50] At this dose, sodium nitrate and potassium nitrate showed no potential genotoxicity, and their

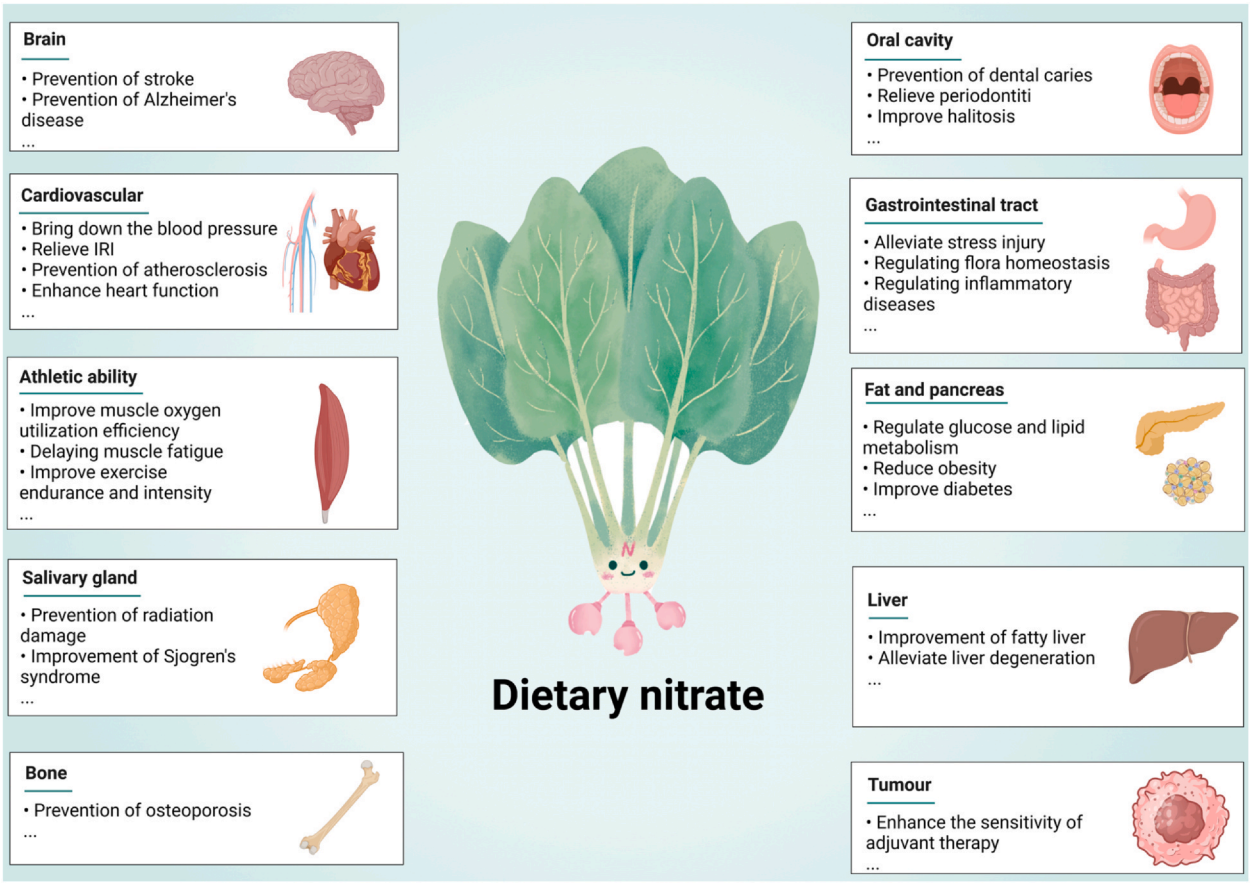

Fig. 8. Physiological function of nitrate. Nitrate has many physiological functions: (1) brain: preventing stroke and Alzheimer's disease; (2) cardiovascular: lowering blood pressure, alleviating ischemia-reperfusion injury, preventing atherosclerosis and enhancing heart function; (3) athletic ability: improve muscle oxygen utilization efficiency, delay muscle fatigue, and improve exercise endurance and intensity; (4) salivary gland: preventing radiation injury and improving Sjogren's syndrome; (5) bone: prevention of osteoporosis; (6) oral cavity: preventing dental caries, relieving periodontitis and improving halitosis; (7) gastrointestinal tract: relieving stress injury, regulating flora balance, and regulating inflammatory diseases; (8) metabolism: regulating glucose and lipid metabolism, reducing weight and improving diabetes; (9) liver: improve fatty liver and reduce liver degeneration; and (10) tumor: enhance the sensitivity of adjuvant therapy. (Created with BioRender.com).

carcinogenicity to rats and mice was negative.[173] To sum up, moderate nitrate intake benefits human health, but excessive intake may bring health risks. In the nitrate intake, you can choose fresh vegetables, fruits, and meat to avoid overeating pickled and smoked food.

7. Summary

Homeostatic balance is a necessary condition for the organism to maintain health, and how to maintain homeostasis is the focus of homeostatic medicine research. Oral health, an essential part of systemic health, is closely related to homeostasis. Nitrate, as a messenger from the oral cavity to the whole body, plays a vital role in homeostasis, and its potential in maintaining health and treating diseases has been widely confirmed. After oral ingestion, nitrate is reduced to nitrite by oral bacteria and reduced to nitric oxide in the body. This process, known as the nitrate-nitrite-NO pathway, can act as a reservoir of NO in the body and exert various biological effects similar to nitric oxide. Sialin, on the other hand, is a nitrate transporter protein present in mammalian cell membranes and intracellular compartments, which can transport nitrate from the bloodstream to the salivary glands and secrete it through the saliva into the oral cavity, where the gastrointestinal tract eventually absorbs it into the systemic circulation, where it exerts its beneficial effects in multiple systems and organs. At the same time, recent studies have found that sialin is present in a wide range of tissues and organs in the body and that interactions with nitrate mediate essential cell biological functions. Nitrate plays a vital role in the maintenance of homeostasis through NO and sialin to maintain the balance of flora homeostasis, inflammatory-immune homeostasis, and energy metabolism homeostasis, which are essential

components for the maintenance of homeostasis in the body to prevent and control multi-systemic diseases, to reduce body damage, to improve athletic ability, to regulate the glucose-lipid metabolism, and to assist in the treatment of tumors.

As a natural dietary nutrient, nitrate has been added to functional beverages for regulating body functions. However, the short half-life and low bioavailability of nitrate in the human body make it difficult to maintain an adequate blood concentration, which is a bottleneck limiting the clinical application of nitrate. Prof. Songlin Wang's group focuses on developing a novel nitrate nanocomposite formulation, Nanonitrator, to overcome this limitation. Nanonitrator has a long-lasting effect, which enables a more sustained release of nitrate and better utilization of their positive impact. In the future, Wang's group will also conduct the necessary safety assessments of Nanonitrator to ensure its reliability in clinical applications. In addition, the mechanism of action of nitrate in living organisms is a complex process involving multiple biochemical reactions and signaling pathways. Although we know some of the nitrate's phenotypic effects in maintaining organismal health and preventing disease, the specific molecular mechanisms are not fully understood. Nitrate and its metabolites can affect cellular signaling through various pathways, and the regulatory agencies of these signaling pathways are complex and require further studies to elucidate. In addition, the nitrate-nitrite-NO pathway involves the catalysis of multiple enzymes and the participation of numerous metabolic pathways, and each step may affect the final effect. Improving the efficiency of NO conversion and reducing the production of harmful substances such as NOC is still challenging.

Interestingly, sialin was previously considered a nitrate transporter responsible only for the transportation of nitrate. However, the latest research of Wang's group shows that sialin exists not only on the surface of cell membranes but also on the surface of organelle membranes and can bind to various classical signaling molecules. It is hypothesized that sialin may be an essential protein that senses and mediates the action of nitrate, which is one of the crucial directions for the future nitrate maintenance of the homeostatic system of the organism.

We have reasons to believe that further application of nitrate in the prevention and treatment of human chronic diseases is promising through in-depth studies of nitrate's role in maintaining body homeostasis. Nitrate, as a messenger of oral intake, will bring more benefits to human health. We look forward to the continuous development of nitrate research, opening up broader prospects for the future medical field.

Conflict of interest

Songlin Wang and Jian Zhou are editorial board members for *Medicine Plus* and were not involved in the editorial review or the decision to publish this article. The authors declare that they have no conflict of interest.

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Author contributions

Huan Liu: Conceptualization, Methodology, Software, Investigation, Formal Analysis, Writing - Original Draft; Jian Zhou: Conceptualization, Funding Acquisition, Writing - Review & Editing; Hideaki Kagami and Lei Hu: Visualization, Writing - Review & Editing; Songlin Wang: Conceptualization, Funding Acquisition, Resources, Supervision, Writing - Review & Editing.

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