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Food and immunity: tackling the diseases of the 21st century

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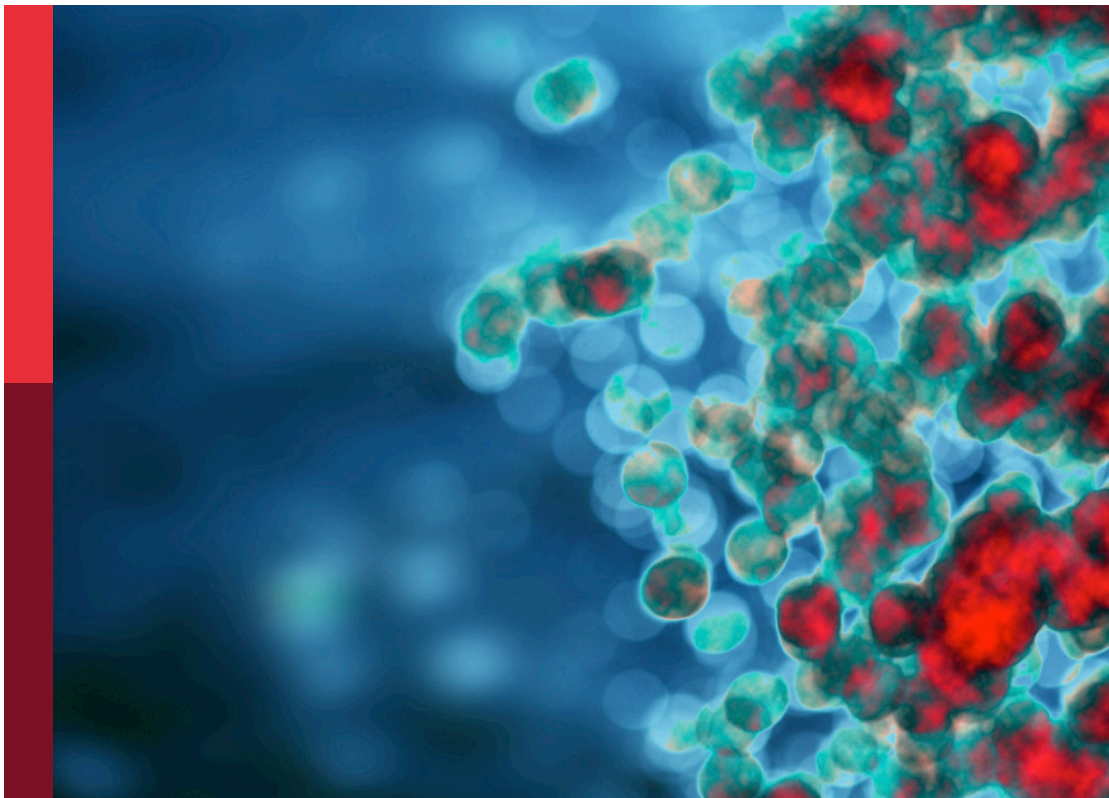

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Food and immunity: tackling the diseases of the 21st century

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Editorial: Food and immunity: tackling the diseases of the 21st century

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Editorial on the Research Topic
Food and immunity: tackling the diseases of the 21st century

Scientific understanding of how diet, gut microbiota, oxidative stress, and immune function interact to influence health has significantly grown over the past 10 years. Recent studies on topics ranging from pediatric health to livestock nutrition and even edible vaccines show that nutritional science, microbiome research, and immunology are increasingly connected. This editorial summarizes key findings from nine studies, offering a clear view of the current state and future research opportunities in human and animal health.

A recent review of the interaction between gut microbiota indicates that diet has a multivariate, bidirectional influence on the structure of the microbiome as well as gut function (1). Shang et al. emphasized the significance of precision nutrition interventions in the promotion of microbial balance and wellbeing. Low-FODMAP diets, individualized probiotics, and even fecal microbiota transplantation are exemplary examples of such interventions (Shang et al.). These findings are in agreement with larger-scale initiatives toward the personalization of dietary recommendations based on the individual microbial and metabolic profiles. This new trend is likely to transform the management of gastrointestinal and metabolic disease (2).

Among the higher-order themes that are conveyed is that of redox homeostasis. Lupu et al. observe that an imbalance between reactive oxygen species and antioxidants is a pathophysiological mechanism in child obesity with impacts on both cardiovascular and metabolic risk. This was supported by previous biomarker research, which showed that obese children experienced decreased glutathione activity with increased lipid peroxidation (3). Two studies using the NHANES database present strong evidence for the protective effect of dietary antioxidants against oxidative stress (4). One of these studies detected a large negative correlation between niacin levels and the incidence of stroke and investigated dietary niacin and stroke risk in adult Americans between 1999 and 2018. Increased intake was seen to reduce the risk of stroke, especially in those with risk factors such as obesity and hypertension. Another NHANES study in 2009–2014 investigated the relationship between periodontitis and the composite dietary antioxidant index. This study detected a direct relationship between nutrition and oral immune modulation, with diets containing high levels of antioxidants such as zinc, vitamin C, vitamin E, and selenium being linked with a reduced prevalence of periodontal inflammation (Meng et al.; Qiu et al.).

Today's studies on probiotics, prebiotics, and postbiotics explore the therapeutic function of diet ingredients regulating gut health in greater detail. Such agents were referred to by the review authors as a "therapeutic symphony" that may coordinate the gut's and immune system's interactions, especially in autoimmune and inflammatory disorders. The review suggests that they work in concert: probiotics enable the re-colonization of healthy microbial strains, prebiotics stimulate their growth, and postbiotics, like microbial metabolites like short-chain fatty acids, modulate immune cell functions. As a package, they are a powerful, non-drug strategy for promoting mucosal integrity and restoring balanced immune responses.

The relevance of the findings goes beyond the domain of human health. Promising results are presented in animal trials on immune modulation with the support of nutritional supplements. Supplementation of diets with vitamins, rumen-protected amino acids, and trace minerals has improved bovine immune capacity, as well as antioxidant and anti-inflammatory processes, especially during the periparturient phase when the risk of mastitis is elevated, as illustrated in a study in dairy cows. These findings emphasize the overlap of immune-nutritional processes across species and indicate that identical methodologies used in human health can be transferred to optimize nutritional interventions in animals.

In widening the scope of investigation, another study investigated the possible effect of dietary cannabinoids and hemp on the health and performance of different animal species. Although the regulatory and mechanistic base is still under development, studies show that cannabinoids, particularly CBD, modulate gut barrier function, inflammatory reactions, and metabolic efficacy in animals. However, there lies the promise of improved agricultural productivity and the development of cannabinoid-derived therapeutic interventions for humans on the basis of the use of plant-derived modulators that influence metabolism and the immune system.

Edible vaccine manufacture is a new method of nutrition-mediated health intervention. A recent milestone study on TOMAVAC, an oral COVID-19 vaccine produced in tomatoes, found that mice and humans generated neutralizing IgG antibodies after eating such genetically engineered tomatoes. This is a cutting-edge convergence of immunology with plant biotechnology and nutritional delivery systems. Edible vaccines can provide the solution to global vaccine problems through reduced reliance on the cold chain and increased availability in resource-poor environments.

Individualized diets are becoming increasingly popular in the context of immunologically mediated disease in children. A study showed that food-specific IgG4-targeted elimination diets decreased considerably symptoms of allergy in children. The findings are in favor of the hypothesis that long-term exposure to food antigens may precipitate immune activation and symptomatology in a subgroup of children with a specific category of pediatric patient, even though there remains some controversy regarding IgG4 testing as a part of allergy diagnosis. The finding contributes to the increasing volume of literature supporting the use of precision diets in the management of inflammatory and allergic disease.

The overlap of these studies highlights shared central themes: that oxidative stress and inflammation are prevalent in many chronic diseases; that diet and manipulation of the microbiome are an attractive, non-surgical therapeutic strategy; and that interdisciplinary, nutrition-focused approaches can enhance the health outcomes in animals and humans. Furthermore, these studies recognize the necessity for a deeper understanding of immune modulation and molecular nutrition to inform the development of plant-based immunotherapies, enhance productivity in livestock, and address childhood obesity.

It is clear that the implications of these results must be accepted in future research. Future research must aim at the integration of multi-omics technologies into clinical and agricultural research, such as metabolomics, redox proteomics, and microbiomics. The translational value of these results could be greatly increased by randomized controlled trials assessing oral microbiome-directed treatments for periodontitis and antioxidant-supplemented nutrition in children. Novel functional foods, such as edible vaccines and cannabinoid-derived animal supplements, also have the potential to be enabled by new safety and regulation paradigms.

Finally, the converging evidence reinforces a new paradigm of agriculture and medicine that views the gut as a key axis of health, prioritizes the quality of diet and antioxidant balance, and employs food as preventive and therapeutic medicine. Today, it is common for nutritional science, microbial ecology, immunology, and biotechnology to intersect. This inter-disciplinary research provides actionable and scalable solutions to some of the biggest health issues of the time.

Author contributions

US: Validation, Writing – original draft, Data curation, Methodology, Project administration, Supervision, Conceptualization, Investigation, Software, Funding acquisition, Resources, Visualization, Writing – review & editing, Formal analysis. BS: Funding acquisition, Resources, Validation, Writing – review & editing, Software, Project administration, Writing – original draft, Formal analysis, Conceptualization, Methodology, Data curation, Visualization, Investigation, Supervision.

Conflict of interest

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Potential of dietary hemp and cannabinoids to modulate immune response to enhance health and performance in animals: opportunities and challenges

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Cannabinoids are a group of bioactive compounds abundantly present in *Cannabis sativa* plant. The active components of cannabis with therapeutic potential are known as cannabinoids. Cannabinoids are divided into three groups: plant-derived cannabinoids (phytocannabinoids), endogenous cannabinoids (endocannabinoids), and synthetic cannabinoids. These compounds play a crucial role in the regulation various physiological processes including the immune modulation by interacting with the endocannabinoid system (A complex cell-signaling system). Cannabinoid receptor type 1 (CB1) stimulates the binding of orexigenic peptides and inhibits the attachment of anorexigenic proteins to hypothalamic neurons in mammals, increasing food intake. Digestibility is unaffected by the presence of any cannabinoids in hemp stubble. Endogenous cannabinoids are also important for the peripheral control of lipid processing in adipose tissue, in addition to their role in the hypothalamus regulation of food intake. Regardless of the kind of synaptic connection or the length of the transmission, endocannabinoids play a crucial role in inhibiting synaptic transmission through a number of mechanisms. Cannabidiol (CBD) mainly influences redox equilibrium through intrinsic mechanisms. Useful effects of cannabinoids in animals have been mentioned e.g., for disorders of the cardiovascular system, pain treatment, disorders of the respiratory system or metabolic disorders. Dietary supplementation of cannabinoids has shown positive effects on health, growth and production performance of small and large animals. Animal fed diet supplemented with hemp seeds (180 g/day) or hemp seed cake (143 g/kg DM) had achieved batter performance without any detrimental effects. But the higher level of hemp or cannabinoid supplementation suppress immune functions and reduce

productive performance. With an emphasis on the poultry and ruminants, this review aims to highlight the properties of cannabinoids and their derivatives as well as their significance as a potential feed additive in their diets to improve the immune status and health performance of animals.

KEYWORDS

animals, cannabinoids, hemp seed, therapeutic potential, anti-oxidant, immunomodulation

Highlights

- Cannabinoids are a class of naturally occurring compounds found in the cannabis plant that plays a crucial role in regulating various physiological processes, in animals.
- Dietary cannabinoids could play a role, in improving appetite regulation, reducing inflammation, managing stress, and promoting overall well-being in animals.
- Certain cannabinoids, such as CBD (cannabidiol), might have the potential to improve feed efficiency in animals.
- Modulating the rumen microbiome through cannabinoids might have broader implications for gut health in ruminant animals, influencing overall well-being and potentially reducing the risk of digestive disorders.
- Dietary cannabinoids serve as a natural alternative to traditional animal health interventions, such as antibiotics or anti-inflammatories.
- Cannabinoids have been observed to exhibit protective effects against challenges posed by endotoxins and lipopolysaccharides.

1 Introduction

The current era of antibiotic resistance has raised concerns about the use of antibiotics in various fields, including animal production system (1). Antibiotics have been commonly used in animal agriculture to promote growth, prevent diseases, and improve feed efficiency (2). In response to the challenges of antibiotic resistance, there has been increasing interest in alternative strategies for promoting animal health and productive performance (3, 4). The use of phytobiotics, which are plant-derived substances with potential health-promoting properties (5). Plant and animal derived additives such as essential oils, plant extracts, and bioactive compounds, offers a range of medicinal benefits including antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory and could be used as alternative to antibiotic and contributing to the overall sustainability of livestock industry (6–9).

Over 480 significant active chemicals have been identified as cannabinoids, the active cannabis-derived compounds with medicinal activity. Each active pharmacological ingredient in a cannabis sample has a different concentration depending on the subspecies of the plant, how the leaves were dried, when the leaves were harvested, the plant's age, and other elements (10). The three main categories of cannabinoids are endogenous cannabinoids (endocannabinoids), herbal cannabinoids (phytocannabinoids), and synthetic cannabinoids. Cannabinoids are chemical substances that primarily act on certain cannabinoid receptors (11). Cannabidiol derived from cannabis plant has gained popularity for its potential therapeutic properties and is being explored in various industries, including agriculture and livestock sectors. The potential application of of cannabinoids in animal feed is a relatively new and expanding area of research. Some studies suggest that cannabinoids may have anti-inflammatory and stress-reducing effects, which could potentially benefit livestock.

Cannabinoid receptors are categorized into two types, cannabinoid receptor type 1 (CB1) and cannabinoid receptor type 2 (CB2), which have been associated to heterotrimeric guanine nucleotide-binding proteins (G-proteins). The effects of cannabinoids on intelligence and thinking ability, hunger, emotions, memory, perception, and motor function are correlated with the widespread distribution of CB1 receptors in the brain central nervous system (CNS). CB2 receptors are more prevalent in the immune system and peripheral nervous system than in the CNS, where they play pivotal role in the control of inflammation and pain (12). Delta-9-trans-tetrahydrocannabinol (δ9-THC), more commonly called "THC", is the psychoactive component of cannabis that makes it a popular recreational drug. A typical cannabis plant's component can contain up to 10% THC. One of the cannabinoid compounds known as CBD is not thought to be psychoactive and has more of a medical use (13).

Tetrahydrocannabinolic acid (THCa) and cannabidiolic acid (CBDa), present in plant during its growth, are converted to THC and CBD by heating process known as "decarboxylation" (14). Based on its cannabinoid content, cannabis is categorized into chemotype I, II, III, IV and V. High levels of the psychoactive compound 9-tetrahydrocannabinol (9-THC) are present in chemotype I, which is utilised therapeutically. Chemotype two characteristics fall between those of fibre and medicinal hemps. Chemotypes three and four are threadlike and have relatively low concentrations of psychoactive chemicals and high concentrations of nonpsychoactive cannabinoids. Chemotype V, the final group, is fibrous and devoid of cannabinoids (15). *Cannabis sativa* L. C.

sativa L. var. *ruderalis*, var. *indica*, var. *sativa* and C. *sativa* L. are the cultivars that are currently considered as one diverse species (14).

Given that scientists are much interested in the potential health advantages of cannabinoids from *Cannabis sativa* L. in the making of food, veterinary medicine, and medicines Table 1. Our goal is to give a particular summary of the latest information regarding the cannabinoids and plant properties, as well as an evaluation of the cannabinoids' potential for usage in food and medicine.

2 History and origin

Cannabis sativa L. is among the planet's earliest cultivars of plants. Initially utilised as a source of fodder in animal feed and as a fabric for clothing, humans eventually turned to it as a source of food and medicines (15, 30). The plant includes cannabinoids, which are bioactive substances (10). Hemp has been used medicinally in Europe since the thirteenth century. Its antiepileptic, palliative, and antiemetic qualities were discovered in 19th century (31). In terms of land use for hemp production and the quality of the items produced by the end of the 1950s, Russia and Italy were the top two countries (32, 33). Canada was among the first nations to legalise industrial hemp production, and it continues to be a major distributor and exporter of the crop, notably in the food business (33). The European Union is the world's 2nd-largest cultivator of Cannabis sativa L., with centres in Romania, the Netherlands, Lithuania, and France. C. sativa L. has long been recognized as an important plant roughage resource. Hemp seeds have acquired admiration over last few years due to their high nutritional contents and presence of phytochemicals that have positive effects on human health (33).

Cannabis sativa L belongs to the Cannabaceae family and the Urticales order. This perennial herb is cultivated in the Boreal Hemisphere's temperate conditions (34). Since the plant has scattered throughout the world and has been changing for generations, it is unknown where hemp first appeared to grow (15, 30, 34). There are records of *Cannabis sativa* L. cultivation and use dating back to the Neolithic era. In cave artefacts from around 700 before Christ (BCE), the first known instances of the plant's use for therapeutic purposes were discovered. The origin of *Cannabis sativa* L. may have been in Central Asia, from which it may have migrated to the Mediterranean region, Eastern, Central Europe, especially in Afghanistan and Pakistan. According to studies, *Cannabis sativa* L. has two additional centers of species diversity; the Hindustani and European-Siberian varieties (35).

3 Potential of cannabinoids to address autoimmune diseases and chronic inflammation

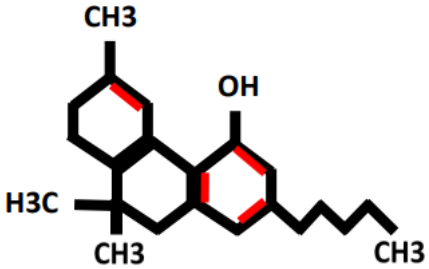
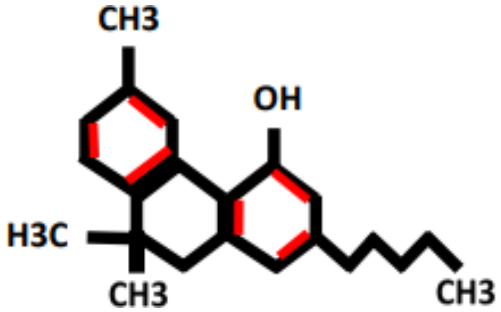
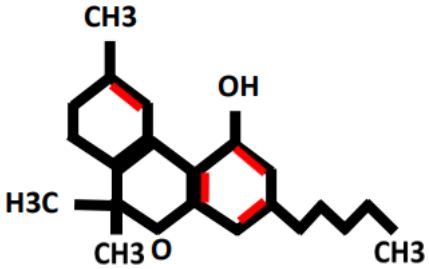
A so-called cannabinoid system made up of certain receptors and ligands appears to exist in the immune system and brain tissues. This system mediates communication between the various tissues, along with others that use hormone and cytokine agents (36). Even though the structure and function of the cannabinoid structure have been extensively studied, there are still many unanswered questions, particularly in regards to the system's role in immunity (i.e., immune cannabinoid system).

3.1 Evidences of cannabinoid receptors in autoimmune system

Cannabinoid receptors (CBRs) can be divided into at least two subtypes, CB1 and CB2. At first, pharmacological evidence implied that these receptors were present in brain tissue and was verified by cloning of CB1 using complimentary DNA from a *Ratus ratus* CNS (37, 38). Interestingly, a human immune cell line rather than the brain was used to clone the second subtype, CB2 (39). It became clear right away that the CBR system existed in immune system cells in addition to the brain cells. CBRs are grouped into 7 transmembrane G protein-coupled receptor super families (40), Moreover, recent studies suggest that they may also bind to Gs proteins, despite the fact that they convey signals via a pertussis toxin-sensitive Gi/Go inhibitory pathway (41). Notably, immune system cell signalling has been connected to G protein pathways (42). The brain and peripheral organs both have endogenous ligands for these receptors in addition to CBRs (43). Because they are structurally based on arachidonic and palmitic acids rather than cannabinoids, these molecules often have a lower affinity for CBRs than cannabinoid derivatives (44). Their existence lends credence to the current hypothesis that the entire cannabis system, which consists of endogenous receptors and ligands, regulates a wide range of physiological processes in both the brain and peripheral tissues. They are created by immunological and brain cells respectively (45).

The discovery of CB1 mRNA expression in human testis tissue provided the first evidence of CBRs being expressed outside of the brain (46). Following this, it was discovered that human peripheral blood mononuclear cells (PMBCs) and mouse solenocyte's both expressed CB1 mRNA using reverse transcriptase polymerase chain reaction (47, 48). Additionally, it was shown that immune cells and the rat spleen expressed the second receptor subtype CB2 at higher levels than CB1 rather than the brain (39, 49). Immune system cells have different levels of CBR expression. For instance, polymorphonuclear neutrophils, B cells, CD8 cells, NK cells, monocytes, and CD4 cells are in decreasing order of CB1 expression in human peripheral blood mononuclear cell (48). Interactions between the cannabinoid systems have lately been found to follow this tendency. The expression of cannabinoid receptors and anandamide in the immune system, brain, and hypothalamic-pituitary-adrenal (HPA) axis has been demonstrated. Both receptor subtypes seem to be expressed by the immune system. Combined with other cytokines and neuroimmune hormones, the cannabis system may facilitate bidirectional communication between neural and immune tissues mouse splenocytes (50). These investigations, along with others, have contributed to the development of the current hypothesis for CBR distribution, which states that CB1 is largely found in brain and nearby structures like the pituitary (51) and peripheral nervous

TABLE 1 Properties of cannabinoids.

NAME OF CANNABINIODS	MOLECULAR STRUCTURES	POTENTIAL HEALTH BENEFITS	ADVERSE EFFECTS	BINDING SITES	REFERENCES
Tetrahydrocannabinol (THC)		Improve sleep and stimulate appetite	Dysphoria,hallucinations,paranoia, drowsiness, uncertainty, headache, xerostomia, depression, elation, low blood pressure, Seizures	Cannabinoid receptor type 1 (CB1) Cannabinoid receptor type 2 (CB2)	(16–18)
Cannabinol (CBN)		Antidepressant	Not studied yet	Cannabinoid receptor type 2 (CB2) G protein coupled receptors (GPCR)	(19)
Tetrahydrocannabivarin (THCV)		Treatment of epilepsy and obesity	vomiting, upper respiratory tract inflammations, serious mental disorders anxiety, depression and suicidal thoughts	Cannabinoid receptor type 1 (CB1)	(16, 20–22)

(Continued)

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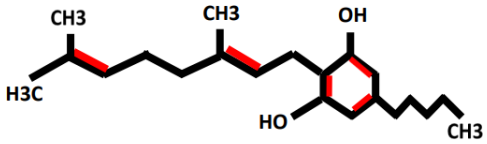
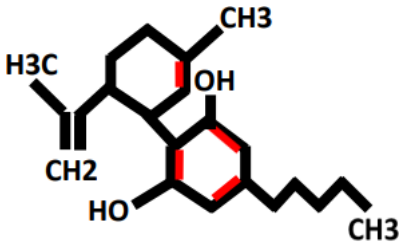
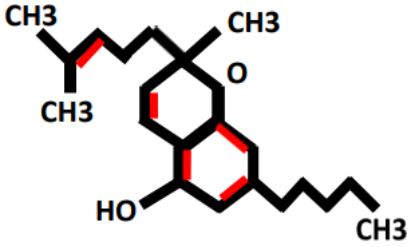
TABLE 1 Continued

NAME OF CANNABINIODS	MOLECULAR STRUCTURES	POTENTIAL HEALTH BENEFITS	ADVERSE EFFECTS	BINDING SITES	REFERENCES
Cannabigerol (CBG)		Antineoplastic	Not studied yet	Steriods like corticosterone and corticol	(23, 24)
Cannabidiol (CBD)		Analgesic Anti-inflammatory Anxiolytic	liver damage, somnolence, sedation, increased suicidal thoughts	Transient receptor potential vanilloid 1 (TRPV1)	(25–27)
Cannabichromen (CBC)		Antidepressant	Constipation inflamed intestine	Transient receptor potential vanilloid 1 (TRPV1) Cannabinoid receptor type 2 (CB2)	(28, 29)

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tissues (52), while CB2 is largely found in the immunological and reproductive systems. Along with the numerous CBR subtypes, these organs also express endogenous ligands such as anandamide. The outcome is the development of the body's immunological cannabinoid system.

3.2 Cannabinoid use in auto immune diseases

Cannabinoids have been tested as a possible treatment for a number of chronic auto immune illnesses. Autoimmune deficiency syndrome (AIDS) and multiple sclerosis (MS) are two of these. The manufacturing of reliable THC chemical formulation and delivery methods that are secure to use and more potent than marijuana smoking is a main problem in the utilization of CBD in these ailments as it is known that smoking marijuana is a fundamental delivery system for THC that also transfer toxic compounds. While inhalers and cutaneous patches are already in the works, THC- and other active cannabinoids-containing medication formulations have not yet been created (53).

3.3 Antitumor effects of cannabimimetic agents

The patterns of hematopoietic and tumor cells development are affected by cannabimimetic substances. For instance, anandamide greatly boosts the proliferative effect of IL-3 on the myeloid cell line 32Dcl3 via a CB2-mediated mechanism (54). Anandamide, on the other hand, prevented the development of breast and prostate cancer cell lines when the levels of prolactin and nerve growth factor receptors were decreased (55). The inhibitory impact was shared by several cannabis agonists, and the CB1 receptor appeared to be implicated hence, these compounds can prevent tumor development in mice and rats (56). To demonstrate this, mice were given THC with other cannabimimetic drugs for up to 7 days following the implantation of C6 glioma cell tumors. This therapy increased survival and reduced tumor size (56). Additionally, it was demonstrated that the drug's mode of action involves causing tumor cells to undergo apoptosis. Numerous research studies, like this one, have demonstrated that substances associated to cannabis cause apoptosis (57, 58). It's likely that the main mode of action by cannabimimetic medicines in a variability of tissues, with malignancies, is programmed cell death.

3.4 Anti-inflammatory effects of cannabinoids

According to recent studies, cannabinoids and their non-psychoactive derivatives have anti-inflammatory potential in addition to their popular usage as analgesics. Oral administration of the THC-11-oic acid dimethylheptyl derivative to mice reduced both short-term and long-term inflammatory changes (59). Additionally, it has been demonstrated that this chemical has potent analgesic and anti-inflammatory properties and is well tolerated by the host when administered orally (59). In multiple studies, it has been shown that the non-psychoactive cannabinoid HU-211 reduces inflammation brought on by the release of cytokines like TNF-a (60, 61). These studies highlight a significant issue with the link between marijuana's effects on cytokines and these chemicals' effects on inflammation. The drug's anti-inflammatory effects are most likely caused by a reduction in cytokine production or activity. According to Klein et al. (62), cannabimimetic drugs have a significant impact on cytokine biology and, depending on the circumstance, may have proinflammatory or anti-inflammatory effects. More study is necessary to settle these possibilities.

4 Role of cannabinoids to control oxidative stress in animals

Oxidative stress as a result of emergence of free radicals have pivotal role in the causing of many ailments e.g., atherosclerosis, rheumatoid arthritis, diabetes, cardiovascular diseases, cancer, chronic inflammation, myocardial infarction, post-ischemic perfusion damage and some degenerative ailments in *Homo sapiens* (63–66). *Cannabis sativa* L. is a best resource of naturally occurring antioxidants and could be utilized in the controlling of oxidative stress. Antioxidants protect the body from the side-effects of free ions, stop the oxidation of molecules, and protects from cell damage (67–69). Now-a-days, much research has been done on hemp (*Cannabis sativa* L.), also known as industrial cannabis which is basically studied because of its chemical composition i.e. one hundred and thirty-three cannabinoids and terpenes (70). Cannabinoids such as tetrahydrocannabinol (THC), cannabinol, and cannabidiol (CBD) are potential lipophilic antioxidants (71), and their pathway of CBD and THC has been reported (72). For many years, researchers have examined and well documented the antioxidative and anti-inflammatory characteristics of cannabis in a range of tissue types and cellular models (73). The antioxidant activity of CBD is seen in Figure 1. Numerous studies have shown that CBD, the main non-psychoactive phyto cannabinoid in *Cannabis sativa*, has a wide range of anti-inflammatory properties and a propensity to control oxidative processes in neuropathic and inflammatory models (74).

4.1 Cannabinoids mode of action to control oxidative stress

CBD has both cannabinoids receptor-dependent and -independent modes of action. It also exhibits very low affinity and negligible agonist activity for both CB1 and CB2 receptors (16, 75). Peroxisome proliferator-activated receptor- (PPAR-) as a CB1/2-independent mechanism of action for CBD (76, 77), TRPV1 receptor (78), G-protein coupled receptor 55 (GPR55) (79), 5-hydroxytryptamine (5-HT) receptors (76, 80–82) and μ-/δ-opioid receptors (Kathmann et al., 2006) are discovered.

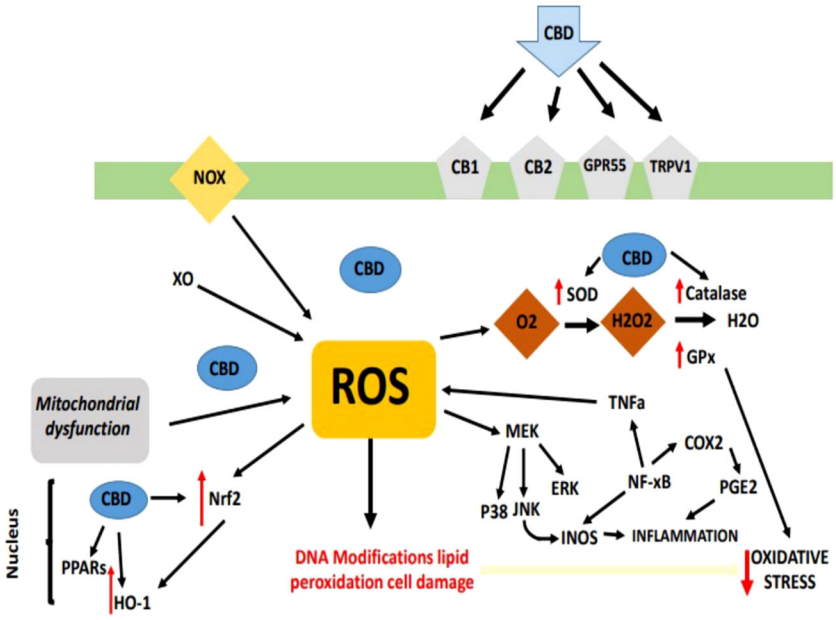

FIGURE 1
Overview of antioxidant property of cannabinoids, especially CBD, have been shown to act as scavengers of free radicals via neutralizing the reactive molecules, preventing them from causing cellular damage.

4.2 Evidences of CBD to control oxidative stress

CBD has been shown to lessen oxidative metabolism in polymorphonuclear leukocytes and nucleus pulposus cells that have been exposed to H_2O_2, and numerous research have suggested that CBD possesses antioxidant capabilities (83, 84), and moreover lowers pancreatic cell oxidative stress markers (85). It's interesting to note that CBD works similarly to vitamin E (alpha-tocopheryl acetate) in reducing the generation of reactive oxygen species (ROS) in the brain after exposure to cadmium chloride (86), additionally, data suggests that it is more neuroprotective against glutamate toxicity than ascorbate and a–tocopherol (86). Due to the physiological and pharmacological variety of CBD and sign of its similar antioxidant activity to identified antioxidants, CBD is a promising medication for therapeutic immunomodulation. Following are the evidences that are collected from various researches that proves role of CBD in oxidative stress Figure 2.

4.2.1 Role of CBD in redox equilibrium

According to a large body of research, CBD alters redox equilibrium via changing the concentration and activity of antioxidant molecules. In fact, research on CBD has demonstrated that it affects how redox-sensitive transcription factors like nuclear factor erythroid 2–related factor 2 (Nrf2) are controlled in microglia (87), keratinocytes (88) and endothelia (89), It is critical because Nrf2 is necessary for cytoprotective and antioxidant gene transcription to begin (90).

Through intrinsic methods, CBD primarily influences redox equilibrium. According to data, CBD breaks up free radical chain reactions and uses the hydroxyl groups on its phenol ring and electrophilic aromatic region to change free radicals into more innocuous molecules (91). It was demonstrated that CBD delivered electrons at a potential similar to that of well-known antioxidants and inhibited hydroperoxide-induced oxidative damage in neurons using the iron-catalyzed ROS production technique (Fenton reaction) and cyclic voltammetry (86). Using cyclic voltammetry once more, it was shown that CBD is an antioxidant on par with tocopherol and butylated hydroxytoluene, two widely used antioxidants (92). Recent evidence showing that CBD can lessen the formation of ROS by chelating the transition metal ions involved in the Fenton reaction (93). According to data, although concurrently amplifying Yo-induced ROS generation, CBD reduces the destruction of mitochondrial membrane potential brought on by anti-Yo antibodies in a way comparable to that of the ROS scavenger butylated hydroxytoluene. This shows that CBD protects against paraneoplastic cerebellar degeneration caused by anti-Yo (94). In an oxygen-glucose-deprivation/reperfusion injury paradigm, additionally, CBD has been shown to guard against energy stress on hippocampus neurons by controlling glucose uptake and triggering the pentose-phosphate pathway (95).

4.2.2 Role of CBD in controlling protein expression

Recent research has demonstrated that CBD can target the expression of Kelch-like ECH-associated protein 1 (Keap1) and Nrf2 in pulmonary artery smooth muscle cells, potentially boosting its antioxidant benefits in a model of pulmonary arterial hypertension (96). Furthermore, CBD regulates the expression of the induced antioxidant enzyme heme oxygenase-1 (HO-1) in keratinocytes (97), adipose tissue-derived mesenchymal stem cells

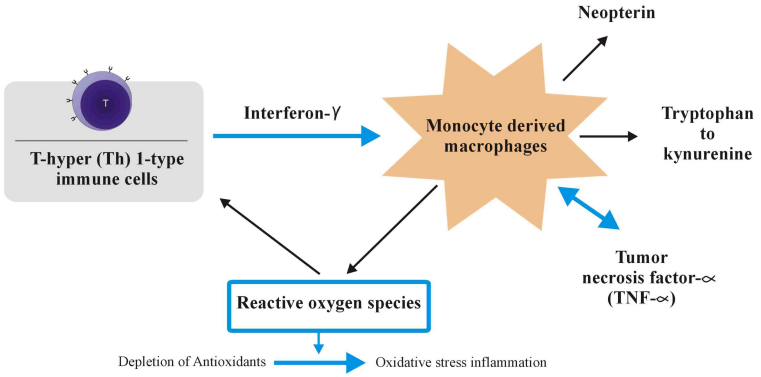

FIGURE 2
During the adaptive immune response, immune cells of the T-helper (Th)1 type that have been activated create cytokines like interleukin-2 or interferon (IFN). In monocyte-derived macrophages (M), IFN-, a pro-inflammatory cytokine, stimulates the production of reactive oxygen species (ROS), as well as the action of indoleamine-2,3-dioxygenase (IDO) and GTP-cyclohydrolase I, which are both involved in the alteration of tryptophan to kynurenine and the production of neopterin, respectively. The creation of tumour necrosis factor (TNF), which increases macrophage receptivity to pro-inflammatory IFN, is triggered by the formation of ROS, which also activate redox-sensitive signal transduction cascades. When cells' antioxidant defences are continuously overwhelmed by ROS, oxidative stress and inflammation result.

(98), neuroblastoma cells (99) and smooth muscle (100). This may have an impact on how effectively this Phyto cannabinoid regulates the level of ROS in cells. In fact, irrespective of CB receptors, in a time- and concentration-dependent approach, CBD dramatically upregulates HO-1 mRNA and protein expression in human umbilical artery smooth muscle cells (89).

4.2.3 Role of CBD in activity of superoxide dismutase

Previous studies have shown that CBD can control the activity of the superoxide dismutase (SOD) enzyme as well as the Cu, Zn, and Mn-SOD enzymes (88, 101). CBD's vasorelaxant effects are diminished by a Superoxide Dismutase (SOD) inhibitor, demonstrating that SOD increases CBD's vascular activities (102). Additionally, by raising glutathione (GSH) levels and concurrently raising GPx and SOD1 activity after injury, CBD reduces hippocampus oxidative damage during oxygen-glucose deprivation/reperfusion injury (95). *In vivo* injection of CBD mitigates the decline in the oxidized glutathione ratio (GSH/GSSG) in diabetic mice's cardiac tissue (101). Further shields against GSH depletion in cardiac tissue after doxorubicin cardiotoxicity (103).

4.2.4 Role of CBD in activity of ROS

Data shows that CBD has an inherent capacity to scavenge free radicals. In fact, it has been demonstrated that CBD reduces LPS's ability to cause ROS in microglia (104). Additionally, CBD inhibits the production of mitochondrial superoxide in human coronary endothelial cells stimulated by high glucose levels and lowers the production of mitochondrial ROS after hippocampal oxidative injury caused by oxygen-glucose deprivation/reperfusion injury (105). In models of retinal neurotoxicity, CBD has been shown to have neuroprotective benefits by directly reducing N-methyl-D-aspartate (NMDA) mediated oxidative stress and maybe by targeting the synthesis of nitro tyrosine, a byproduct of tyrosine nitration (106). Similarly, it has been demonstrated that CBD has ROS scavenging properties created by H_2O_2-driven ROS in keratinocytes and oligodendrocyte progenitor cells, shielding them from H_2O_2-induced cell death (107, 108). Recently, it was demonstrated that CBD had a comparable impact on H_2O_2-induced ROS in intestinal cell monolayers (109). Furthermore, findings show that CBD works similarly to -tocopheryl acetate in reducing brain ROS generation after exposure to cadmium chloride (110). Moreover, CBD dose-dependently lessens the generation of ROS in neurons caused by β-amyloid (111). Parallel to this, CBD has been demonstrated to lessen cisplatin's induction of renal nitro tyrosine synthesis in a model of nephrotoxicity (112). It has additionally been demonstrated to dose-dependently decrease the ROS generation brought on by tert-butyl hydroperoxide in keratinocytes (97). Accordingly, polymorphonuclear leukocytes exposed to chemotactic peptides produce less ROS when CBD is present (83) additionally, administering CBD *in vivo* reduces the level of lipid peroxides and ROS in diabetic mice's cardiac tissue (113). Last but not least, new study by Baeeri and colleagues (85) demonstrates that CBD can serve as a free radical scavenger in response to a range of stressors by decreasing age-related increases in ROS production in pancreatic islets.

5 Nutraceutical effects of cannabinoids

Hempseeds and seed meal derived from Cannabis sativa have proven to be significant contributors to the Old World's food resources. These seeds are abundant in essential fatty acids, such as omega-3 and omega-6, making them a nutritious source of dietary oil. Moreover, they offer a substantial amount of protein and fiber, enhancing the overall balance on nutrients and bioactive compounds. The prospective usage and advancement of *Cannabis sativa* seed as a source of nutrition for human and house animals

was halted after the forbidding of Cannabis variants growth in the late 1930s (114).

Whole hempseed typically contains 20 to 25 percent protein, 25 to 35 percent carbohydrates, along with 10 to 15 percent insoluble fibre, and 25 to 35 percent oil (taken via cold pressing the seeds or by extraction of oil) (114, 115). Regarding the nutraceutical abilities of cannabis by-products, various outcomes of their inclusion to basal feed have been hypothesized, includes a decrease in the occurrence of tibia deformation in egg-laying chicks and hens, an enhanced serum lipid profile, a protective impact against the onset of hepatic disease, an anti-microbial activity, an improvement in anti-oxidative systemic condition, and an anti-inflammatory action (116–120). However, additional work and study is required to establish all of these beneficial effects.

6 Effect of cannabinoids on nutrient digestibility, feed efficiency and live weight gain

The overall digestibility of feed or distinct nutrients is precisely known as the amount or percentage that is not eliminated in fecal waste hence considered to be retained by the organism. There were no negative impacts on digestibility due to the existence of any secondary compounds in *Cannabis sativa* straw (121). However, it is unclear why the digestibility of dry matter (DM) and organic matter (OM) has improved. In comparison to the hemp-containing pellets, the control diet exhibited elevated concentration of polyphenolic chemicals. Polyphenolic compounds such as tannins diminish the digestibility of food by binding to gastric enzymes and dietary proteins, as compared to a controlled diet (122). Hemp contains flavonoids, which can lower DM digestibility (123, 124). Digestibility and lignin contents are inversely associated (125, 126). When hemp stubble was added to the pelleted diets, the lignin content increased, but there was no negative correlation between digestibility and lignin level. The digestibility of a diet is also impacted by variations in the neutral detergent fiber (NDF) and digestibility of the forage products. Oat straw typically has an NDF digestibility of above 20 percent (127), in contrast to *Cannabis sativa* stem which is 12.7 percent (121), suggesting that oat straw could be more easily digested than hemp stalk. More research into the digestibility of *Cannabis sativa* straw is necessary to understand the changes in apparent DM, OM, NDF, and Acid Detergent Fiber (ADF) digestibility's as a result of *Cannabis sativa* straw addition in the pelleted diets. In order to study, cannabinoid role in controlling feed conversion ratio, cold-pressed *Cannabis sativa* seed cake was studied as a protein feed for young cows and finishing steers. Effects on feed intake, live weight gain (LWG), faecal traits and carcass traits (steers only) were investigated. Animals fed Cannabis sativa seed cake consumed more NDF than those fed Glycine max diet ($P < 0.05$). Lower feed efficiency as a result of higher feed intakes and equivalent LWG was observed in calves given *Cannabis sativa* ($P < 0.05$). In summary, developing cattle who are aggressively fed *Cannabis sativa* seed cake instead of *Glycine max* meal produce equivalent amounts of milk and have better rumen functions (128).

7 Role of cannabinoids in nutrient absorption, metabolism, and excretion

The ECS clearly plays a critical effect in macronutrient metabolism, hence regulating feed consumption and body energy homeostasis (129, 130). General pathway illustrating role of THC in energy metabolism is given in Figure 3.

7.1 CB1 activation stimulates appetite and nutrient uptake

CB1 promotes intake in animals by causing orexigenic peptides to bind to hypothalamic neurons and preventing the addition of anorexigenic proteins (131). After feeding, the adipose tissues (AT) releases leptin hormone in this metabolic process, which binds to the hypothalamus and causes the release of anorexigenic peptides (132). According to studies, leptin resistance and hyperleptinemia in a diet-induced obese mouse model were reversed by peripherally-restricted CB1 inverse agonist (133). These findings show how CB1 can inhibit the hypothalamic leptin sensitivity and satiation signaling pathways thus playing pivotal role in nutrient uptake (134).

The gastrointestinal tract contains all of the components of the ECS. When food is first taken into the mouth during a meal, cephalic-phase reactions happen to anticipate and prepare for optimal digestion. The orexigenic hormone ghrelin, which is released when the gastric CB1R is activated, raises the perception of fat and encourages consumption of fat (135). Furthermore, in both rodents and humans, the ECS in the gut may change cholinergic transmission to the colon, lowering intestinal motility (136). Additionally, the CB1Rs' anti-inflammatory properties make the ECS a possible enhancer of food absorption in the GI tract (136).

7.2 NAPE-PLD, the intestinal barrier, and nutrient absorption

Nutrient absorption in rumens is increased by improving gut epithelial barrier and microbial function are affected by adipose tissue levels of N-acetylphosphatidylethanolamine phospholipase D (AT NAPE-PLD), which in consideration enhances energy storage function in a periodic way (137). The intestinal epithelium has a pivotal role in the absorption of nutrients, hormone release, and synthesis of endocannabinoids (eCBs), all of which affect metabolic activity (138). Few minutes' nutritive fatty acids (FA) exposure in the stomach in monogastrics causes jejunal anandamide (AEA) mobilization and FA transport into the duodenum, which enhances oleoylethanolamide (OEA) production (139). Endocannabinoid system (ECS) activation in the stomach enhances adipogenesis in addition to enriching eCB production (140). The intestinal ECS lowers LPS transferring, barrier breakdown, gut inflammation, and dysbacteriosis of gut microorganisms in monogastric animals (140).

When released from the rumen epithelium, lipopolysaccharide (LPS) in dairy cows crosses the intestinal barrier and enters the

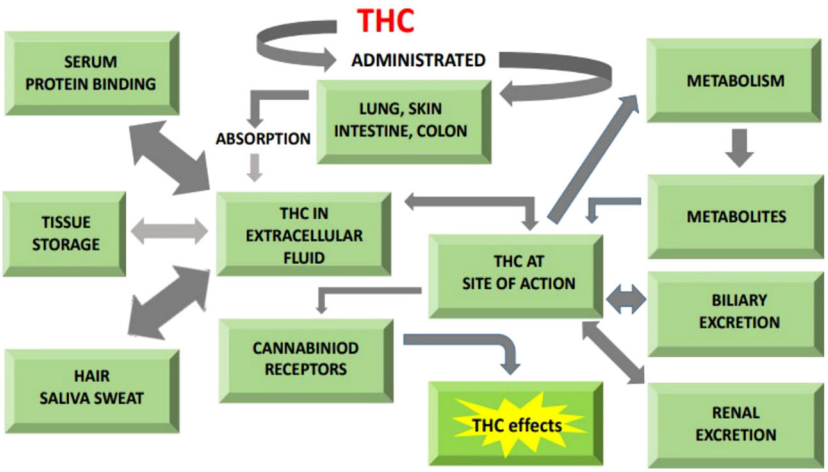

FIGURE 3
General illustration of pathway showing cannabinoids role in absorption, metabolism and excretion.

bloodstream. Elevated levels of endotoxin in the bloodstream lead to substantial changes in metabolism and provoke systemic inflammation (141). The same study found that circulating LPS levels related to blood glucose and non-esterified FA levels (141), and these gains are followed by dairy cows consuming less dry matter (142). It is interesting to note that local CB1 activation reduces the amount of LPS that enters the body, which may increase appetite and reduce inflammation in milking cows.

8 Role of cannabinoids in lipid metabolism

One of the most major health issues in Western countries is obesity, and the discovery that the endocannabinoid (EC) system is involved in the control of energy balance and the focalization of fatness is a huge improvement in our knowledge of this issue. Ancient medicine was aware of the impact of plant-extracted CBD on individual weight or body mass and appetite, but it wasn't until recently that the mechanisms underlying these effects were understood. This was made feasible by the exact EC receptors' identification as well as the endogenous ligands anandamide and 2-arachidonoylglycerol (2AG) (38, 143–145). Numerous experimental studies have shown that ECs are present in adipose tissue and other membrane tissue involved in the energy metabolism. This information provides another hint to understanding adipose tissue function in *Homo sapiens* obesity (146, 147).

Multiple evidences suggest that endogenous cannabinoids are appropriate for the membranous control of lipid management in fat tissue, which follows the revelation that these molecules are taking part in hypothalamus regulation of food intake (147–149). Rimonabant, a sepecific CB1 blocker, has been the subject of numerous phase-III clinical trials, all of which have demonstrated that inhibiting CB1 lowers body mass in fat specimens and improves cardiovascular risk elements in obese and diabetic patients (150–153). A sufficient amount of both fatty acids and glucose must reach fat cells in order to store and expand triglycerides. To feed lipid substrates to fat cells, fatty acid flux from chylomicrons and very-low density lipoprotein is mediated by lipoprotein lipase (LPL). The crucial processes of creating the glycolytic intermediate a-glycerophosphate required for triglyceride synthesis are insulin-dependent glucose transporter (GLUT4) translocation and glucose transport. In both of these pathways, insulin is in charge. Enough insulin sensitivity and the activation of its downstream machinery are thus necessary to permit adequate fuel channeling to fat cells (154).

CB1 receptor is not found on preadipocytes however, upon differentiation, adipocytes rapidly exhibit its expression. This has been seen in both primary *Homo sapiens* adipose cell and primary cells and cell lines from rodents (155). It is debatable whether adult adipocytes express CB2. While some scientists discovered considerable expression of CB2 in differentiated adipocytes, others were unable to (146). It is plausible that predispose cells, invasive macrophages, or vascular cells are the source of CB2 mRNA in fat tissue extracts because CB2 is expressed at modest levels in fat tissue biopsies as well (146). Adipose tissue and fat cells both express CB receptors as well as the enzymatic machinery needed to create and break down endogenous cannabinoids locally (156, 157). In primary mouse adipocytes, activation of CB1 increases lipoprotein lipase activity (158). As a result, there would be a greater inflow of free fatty acids into adipocytes for the synthesis of triglycerides. They found that the strong CB1 agonist HU210 stimulates the creation of intracellular lipid droplets in 3T3-F442A cells, demonstrating the importance of CB1 and ECs in the growth of neutral lipids in fat cells (156). Adipocytes produce more 2AG and anandamide before adipose cell differentiation occurs, supporting the idea that this system is responsible for causing preadipocytes to convert to adipose tissue (156).

The entrance of glucose into fat cells is also encouraged by CB1 activation. CB1 activation increases glucose absorption in human primary adipose cells, and this action is achieved by Glucose

transporter type 4 (GLUT4) moving from an intracellular compartment to the plasma membrane, which is where it is located. Additionally, the cannabinoid-stimulated glucose uptake in fat cells is mediated by the same molecular mechanism as drives insulin-induced glucose uptake stimulation of PI3-kinase. Actually, the benefits of CB1 activation on glucose absorption are totally negated by the inhibition of this enzyme by wortmannin. Additionally, the absorption glucose into the fat cells is mediated by an increase in intracellular calcium from the surrounding environment (146). In studies conducted in calcium (Ca) free medium or with the Ca chelating reagent ethylene glycol tetra-acetic acid (EGTA), The translocation of GLUT4 and the absorption of glucose were unaffected by CB1 activation. Rimonabant fully offset the effects of the CB agonist on glucose absorption. The extent of the ECs' impact on glucose absorption was between 40 and 50 percent that of insulin. However, it is uncertain what physiological consequences EC-induced glucose clearance by fat cells would have. Although the EC's effect as insulin in fat cells is expected to be important for triglyceride accumulation and preadipocyte formation, the influence on the body's ability to handle glucose should be minimal. Rimonabant-based *in vivo* investigations have repeatedly demonstrated that provoking CB1 did not degrade insulin resistance in obese people, instead causing weight loss and a reduction in the size of fat tissues and perhaps adipose fat cells increased whole-body insulin sensitivity (159).

Mice lacking CB1 receptors (CB1/) are thin and unaffected by high-fat diet (160). Similar to how rimonabant, a selective CB1 receptor antagonist, causes reductions in body weight of obese rats over time, however after a brief initial 1–2-week weight loss, food intake returns to normal (160, 161), indicating that the stimulation of energy metabolism by CB1 receptor blockage results in a reduction in fat content. If we take a holistic view of the body, this situation may result from higher energy expenditure along with enhanced oxidative capability of many tissues, in specific the brown adipose tissue, the liver and skeletal muscle. This might be explained by at least three causes if just white adipose tissue is taken into account: The first three alterations are an increase in lipolysis, a decrease in liposynthesis, and an increase in fatty acid oxidation inside the fat cell. Several pieces of evidence show that the CB1 blockage increases lipolysis *in vivo*. A single-dose study on postprandial rats revealed an instant rise in free fatty acids (FFAs), conclusively demonstrating an underlying pharmacological impact of rimonabant to induce lipolysis instead of a secondary one brought on by a decline in intake and after-starvation post-absorptive metabolic alterations in intermediate metabolism (162).

In conclusion, several evidence unequivocally demonstrate that EC and CB1 receptor levels increase during adipocyte differentiation (156, 163, 164). CB1 activation causes pre-adipocytes to differentiate more quickly (156). We propose that elevated lipogenesis is a result of the EC system's overactivity stimulating Lipoprotein lipase (LPL) activity (158), an improvement in insulin sensitivity, as well as a faster rate of glucose absorption and utilization (146, 164) and a fatty acid synthase activation (147). The AMP-activated protein kinase (AMPK) and eNOS-dependent mitochondrial biogenesis in adipose tissue are also inhibited by this overactivation, which reduces ATP generation and oxidative metabolism of energy sources. This process may be reversed by blocking the adipose CB1 receptor, which would reduce adiposity and weight growth. This would provide rimonabant with a fresh, as-yet-unknown mode of action for reducing body weight (165).

9 Potential of cannabinoids to modulate rumen microbiome to enhance expression of fibrolytic genes

The phrase "microbiome" refers to the collective genome of microbial communities, or "microbiota," which are connected to people, animals, and plants. The influence of microbial communities in determining the host immune system and fitness has come to light in recent years (166). There are similarities between the control of host gene expression by the gut and root microbiota (167, 168), catabolic genes that increase their hosts metabolic capabilities (169, 170), and the control of dangerous pathogens (171).

Ruminants account for a sizable portion of all domesticated animal species in the world and the fundamental producers of milk, meat and other by products. Ruminants are able to digest enormous number of plant polysaccharides because of the variety of bacteria that can be found in the rumen. The rumen, which is home to a range of microorganisms like as bacteria, archaea, fungi, viruses, and protozoa, has evolved into a prolific fermentation vessel for the breakdown of cellulose (172, 173), they interrelate and importantly affect ruminants health. Around 95% of all rumen microorganisms are bacteria, which rule over the diverse domains of the rumen's microbiome (174). Microbes play a key role in the rumen fermentation process, which changes the content and quality of milk and meat as well as the productivity of the animal (175).

To break down the intricate plant polysaccharides, rumen microorganisms create a variety of fibrolytic enzymes known as Carbohydrate-Active Enzymes (CAZymes), which include exoglycanases, glucosidases, endoglucanases, and hemicelluloses. Technologies for high throughput sequencing (HTS) are widely utilized to tackle the complex procedure of lignocellulose breakdown in ruminants. With a greater knowledge of the rumen microbial population, In the cattle industry, issues with ruminant nutrition and environmental issues may be tackled. Number of metagenomics investigations have documented different types of fibrolytic enzymes found in the rumen of yak's, reindeer, Jersey cow, Angus cattle, and buffalo (173, 176–178). In-depth scholarly studies on metagenomic analysis on CAZymes profile in rumen of Holstein-Friesian crossbred cattle feeding with just finger millet straw are not yet available, though.

The rumen is a special natural environment due to the genetic diversity of fibrolytic enzymes from microbial origin that break down plant polysaccharides. An investigation was conducted to determine the main cell wall-degrading enzymes in plants and the associated rumen microbiomes taxonomic profiles (179). Through a comprehensive metagenomics sequencing method, the rumen microbiota of cattle and the carbohydrate-active enzymes were

divided into functional groups. The candidate genes encoding fibrolytic enzymes from various classes of carbohydrate-binding modules, glycoside hydrolases, polysaccharide lyases, carbohydrate esterases, glycosyltransferases and auxiliary activities were found through analysis of the assembled sequences using the carbohydrate-active enzyme analysis toolkit. A large fraction of the CAZymes were produced by bacteria from the genera *Prevotella, Fibrobacter, Bacteroides, Clostridium*, and *Ruminococcus*, according to phylogenetic analysis of the contigs that encode the CAZymes (179). The findings showed that the CAZymes and the rumen microbiome of cattle are extremely complex, structurally related, but different from those of other ruminants in terms of content. The rumen microbiota's distinctive traits and the enzymes produced by the residing microorganisms provide chances to increase ruminants' feed conversion efficiency and function as a repository for crucial industrial enzymes for the synthesis of cellulosic ethanol (179).

10 Potential of cannabinoids as a feed additive to enhance animal performance

The European Food Safety Authority (EFSA) panel on Additives and Products or Substances used in Animal Feed stated in its scientific opinion that hempseed and hempseed cakes might be used in animals feed, though there may be differences in rate of incorporation in diet depending on the specie (180). Animal feed may be supplemented with hemp oil, a rich source of vital fatty acids, meanwhile seeds and hempseed cakes can serve as protein and fat sources. The hemp plant produces cannabinoids, terpenophenolic compounds that are closely related to the pharmacological effects of cannabis (181). The bract covering the seed is where hemp has the most THC and other cannabinoids (182). Cannabinoids may be present in hemp seed products in substantial amounts if the hemp seed varieties are not carefully chosen, grown, processed, and handled. For instance, during cold pressing, cannabinoids can be absorbed by hemp seed oil. Cannabinoids from the resin of the flowers or leaves can also be transferred to the seeds during processing and handling.

Cannabis sativa, with the exception of the seeds and roots, produces cannabinoids in glandular organs (trichomes) that are dispersed across the whole surface of the plant. Trichomes are heavily concentrated in the area of influorescence, in the veins of the leaves, and on the sides of the leaves. They contain essential oils, highly polymeric phenols, terpenes, waxes, and resin that contains 80 to 90% cannabinoids. Delta-9-tetrahydrocannabinol (THC), the primary psychoactive substance, is primarily present in the inactive precursor form delta-9-tetrahydrocannabinol acid (THC-A), which may account for up to 90% of all cannabinoids in hemp plants produced in Europe (183). Cannabinol (CBN) and cannabidiol (CBD) are the other two key active ingredients among the 60 additional cannabinoids that have been found. *Cannabis sativa* phenotypes can be identified by their THC + CBN/CBD ratio. The ratio of hemp types grown for fibre production is less than 1, whereas variants grown for cannabinoids show a ratio greater than 1 (184). The plant's cannabinoid content fluctuates according on its vegetative state of development, cultivation conditions (temperature, humidity), and other factors.

When the hemp leaf is used as forages (for cattle, for example), whether in whole or in part, the animals may be exposed to THC at levels higher than those resulting from consumption of the top portion of the same variety classified and assessed for control under the same regulation. In terms of hemp seeds, it has been demonstrated (185), that the majority of THC was discovered on the outside of the seeds due to contamination with plant debris, probably as a result of physical contact with the plant leaves during processing. Numerous research has examined the effects of consuming hempseed or its derivatives on farm animals, albeit the outcomes were not every time obvious. Here is a summary of the most telling research, broken down per animal species.

11 Protection against endotoxins and lipopolysaccharide's challenge

Immune challenges include several pathophysiological situations including stress, endotoxemia, and inflammatory illnesses, which affect how neuroendocrine factors are produced and released normally (186). Lipopolysaccharide (LPS), a gram-negative bacterial endotoxin is an effective inducer of catecholamines, prostaglandin and proinflammatory cytokines to be released (187). It is therefore widely employed to elicit immunological challenge, which in turn affects neuroendocrine systems. During systemic infections, proinflammatory cytokines react to peripheral signals and cross the blood-brain block or fenestrated capillaries in specific areas of the brain to enter the central nervous system (188, 189). Additionally, the brain produces cytokines in the presence of other cells, primarily astrocytes and microglia, but also neurons and endothelial cells (190).

The primary center that receives a multitude of peripheral signals is the hypothalamus because it is the area of the brain where the majority of neuroendocrine factors that control essential pathophysiological activities are produced. In actuality, infectious organisms, antigens, and the LPS challenge quickly engage the immune system, causing it to produce interferon gamma and cytokines that are subsequently transported into the brain where they influence the function of the hypothalamus (191). It is widely known that the release of corticosterone from the hypothalamic-pituitary-adrenal axis, which is activated by the immunological response, regulates the cardiovascular, metabolic, neuronal, and immune systems (192, 193). Last but not least, glucocorticoids create a negative feedback loop that controls both their own production and the immune system (194, 195). It's important to know that endocannabinoid signaling appears to be tightly linked to the proper operation of the hypothalamic-pituitary-adrenal axis (196–199).

The role of the ECS in innate reactions in case of inflammation and brain functions is particularly intriguing (200, 201). Numerous studies have demonstrated that various organs and tissues produce

endocannabinoids as a result of infection and inflammation (202), which act as moderators to control the activated neuroimmune response (203). The profiles of endocannabinoids change significantly during a variety of pathological circumstances, such as Parkinson's and Alzheimer's disease, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), traumatic injury, stroke, and bacterial and viral infections of the central nervous system due to the inflammation-modulating and the way these substances work to reduce pain (204). Additionally, endocannabinoids influence neuroendocrine function. Endocannabinoids must be produced "on-demand" for neurotransmission to be fine-tuned under both resting settings and immunological challenges that regulate the release of neuropeptides, neurotransmitters, and hormones. The formation of ECS differs based on the desired response in both circumstances, and the ECS functions as a intermediary for the transmission in between glial cells and neurons to produce the most feasible neuroendocrine responses in every scenario respectively (205).

12 Effects on poultry health and performance

12.1 Broilers

After C. sativa seeds were added to a basal diet at rates of 10 and 20 percent, broilers' body weight dramatically increased when related to animals fed with the basal feed alone. In contrast to the control group, animals fed a diet containing hempseed had a lower feed intake and a higher feed conversion rate. The higher hempseed content resulted in the best growth performance. In contrast, the broilers body mass was considerably lower than in the control group at hempseed concentrations lower than 5 percent (206). Mahmoudi et al. (116), also observed a decrease in average daily intake and growth in broilers given 2.5 percent hempseeds over the first twenty-one days of treatment, but no change was noted in weight gain with diets at 4 and 7.5 percent (117). Neither hemp oil up to 6 percent nor *Cannabis sativa* seed cakes at 10 percent and 20 percent improved the development performance of hens in the experiments (207, 208). Effects of adding 5 and 15 percent *Cannabis sativa* seed cakes to broiler diets were investigated. Comparing the greater dose to diets without *Cannabis sativa* seed cakes, the researchers discovered a detrimental effect on broiler development but no variations in carcass weight or the ratio of breast to thigh meat (209).

12.2 Layers

The majority of authors came to the conclusion that adding hemp products to chicken diets had no detrimental effects on the bird's performance. Several research has also looked into how adding hemp to eggs affected their levels of saturated fatty acid (SFA) and monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and essential fatty acids (EFAs). The concentrations of linoleic acid (LA) and α-linolenic acid (ALA) increased linearly with the addition of 5, 10, or 15 percent *Cannabis sativa* seed cakes to the diet (210), while SFA and MUFA levels decreased. Neijat et al., 2016 examined the addition of *Cannabis sativa* seeds (ten, twenty and thirty percent) and *Cannabis sativa* seed oil (4.5 and 9.0%) and discovered that the highest amounts of *Cannabis sativa* seeds and *Cannabis sativa* seed oil significantly increased the amount of ALA and docosahexaenoic acid (DHA) in egg yolks when compared to a control group.

The fatty acid profile of egg yolks changed in the study by depending on whether *Cannabis sativa* oil or *Cannabis sativa* seed cakes were included in the diet: While ALA was greater than the control and lower than the Cannabis sativa seed group, LA was higher with Cannabis sativa seed cakes than with Cannabis sativa seeds and the control group. Oleic acid levels in the yolk were lower and MUFA concentrations were lower when chickens were given both hemp derivatives (211). The same study also found that eggs from laying hens fed a diet enriched with hempseeds or hempseed cakes contained higher levels of -tocopherol, indicating a higher antioxidant potential (211). Last but not least, it was discovered that including 25 percent *Cannabis sativa* seed in the diet of hens enhanced the -6/-3 ratio in egg yolks. Up to 12% of laying hen diets could contain hemp oil without having a negative impact on performance metrics or the flavor and aroma characteristics of cooked eggs (208, 212).

13 Effects on health and performance of ruminants

The effects of include hempseed cakes at variable amounts (143, 233, and 318 g/kg dry matter) in the diets of dairy cows were evaluated. When the cows received an increment of 143 g/kg in comparison to the control group animals, who were given hempseed oil, their milk production rose (213). The rate of dietary crude protein conversion into milk protein declined as hempseed cake consumption increased, which prompted the authors to draw the conclusion that adding 233 or 318 g/kg had no positive effects on milk performance (214). In contrast to cattle fed "normal diets," other studies found no differences in weight gain when whole hempseeds or hempseed cakes were included to the diet (213, 215). However, hempseed meal might be regarded as a superior naturally occurring rumen crude protein (216). All things considered, findings suggest that hempseed cakes have better rumen performance than control diets, perhaps as a result of their higher fiber content and lower starch content (215). Studies have shown that including hempseed oil in a hay-based dairy goat diet at a rate of 4.70% increased the milk fat content, while increasing conjugated FA and PUFA proportions, but it had no effect on milk yield (217).

14 Potential of cannabinoids to modulate metabolic signaling pathway

In the brain, 2-Arachidonoylglycerol (2-AG) is present at a baseline level that is roughly 1000 times greater than AEA. Altering

the metabolism of 2-AG, but not AEA, through pharmaceutical means has a notable impact on endocannabinoid-mediated retrograde signaling. These data lead to the hypothesis that the central nervous system's (CNS) have naturally occurring ligand for cannabinoid receptors (CBRs) is 2-AG (218–220). AEA, however, has been demonstrated to independently activate transient receptor potential vanilloid 1 (TRPV1), inhibit l-type Ca2+ channels, and negatively regulate 2-AG production and physiological consequences in the striatum, highlighting its critical function in the control of synaptic transmission (221).

Depolarization-induced suppression of inhibition (DSI)/ excitation (DSE) was the first conclusive evidence for retrograde endocannabinoid signaling (222). Furthermore, it was demonstrated that both short-term depression (STD) and long-term depression (LTD) include activation of endocannabinoid system in excitatory and inhibitory synapses (223, 224). In most situations, increasing intracellular Ca2+ concentrations and active Gq/11-coupled receptors trigger the synthesis of 2-AG, which then initiates endocannabinoid-mediated retrograde signaling (224). Then, by a procedure that is not completely understood yet, before reaching the presynaptic terminal and interacting with the CB1R, 2-AG is entered into the extracellular space and travels through it. Activated cannabinoid receptor 1 (CB1R) reduces neurotransmitter release by inhibiting voltage-gated Ca2+ channels, which reduce presynaptic Ca2+ influx, and adenylyl cyclase (AC) and the subsequent cAMP/PKA cascade, which is implicated in LTD (222–224). 2-AG must be degraded by monoacylglycerol lipase (MAGL), which inhibits signaling by being expressed in certain synaptic terminals and glial cells (223–225).

It has been demonstrated that AEA plays a variety of roles in endocannabinoid-mediated synaptic transmission (Figure 4). TRPV1 is a complete agonist of AEA, and it is thought to play a role in endocannabinoid signaling (218). The effect of AEA's negative regulation of 2-AG metabolism can be mirrored by TRPV1 activation (226). A tonic function for AEA as an endocannabinoid is also supported by the fact that chronic fatty acid amide hydrolase (FAAH) blocking causes persistent agonist of the endocannabinoid system without lowering CB1R appearance, which is the opposite of MAGL antagonism (227). Independent of the type of synaptic transmission or the length of the transmission, endocannabinoids play a significant role in suppressing synaptic transmission through a variety of methods (223, 224). A subset of neocortical interneurons, pyramidal neurons, and hippocampal cornu ammonis (CA1) neurons, as well as CB1R-dependent self-inhibition in postsynaptic neurons, have all been identified (228–230). The ability of microglial cells and astrocytes to make 2-AG or AEA has been demonstrated in earlier research, it is currently unknown, nevertheless, whether these endocannabinoids are involved in the control of synaptic transmission (231). However, despite studies demonstrating the existence of cannabinoid type 2 (CB2R) in the brain, it is still largely unclear how CB2R contributes to endocannabinoid-mediated synaptic transmission (232–234).

The CB1R modifies the working of various ion channel types (235, 236). In cultured *Ratus ratus* primary hippocampal neurons, mouse cerebellar slices, and neuroblastoma cell lines, CB1Rs have been shown to block N-type Ca2+ channels (237–239). It has hypothesized, but only recently demonstrated, that the CB1R controls Ca2+ inflow to reduce the release of y-aminobutyric acid (GABA) in mouse hippocampus slices by altering the activity of presynaptic N-type Ca2+ channels (240). CB1R has been demonstrated to adversely regulate a variety of Ca2+ channel subtypes, including P/Q-type and R-type Ca2+ channels (241). But when CB1R complementary deoxyribonucleic acid (cDNA) is injected into transfected AtT-20 cells, Mus musculus nucleus accumbens slices and rat sympathetic neurons, the CB1R activates GIRK and triggers the activity of G-protein-coupled deeply changing potassium ion channels (242, 243).

Previous research has demonstrated that stimulation of the CB1R causes the extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun N-terminal kinase (JNK), mitogen-activated protein kinase (MAPK) and p38 signaling pathways, which have vital role in the regulation of cell cycle control, cell proliferation, and cell death to become active in a system that expresses the receptor endogenously or heterogeneously (235, 236, 244). The way that CB1R modulates MAPK signaling typically depends on the cell type and ligand (235). For instance, depending primarily on the microenvironment and stimulus type, CB1R-induced ERK1/2 activation can be mediated by G protein, β-arrestin, or phosphatidylinositol-3-kinases (PI3K) (245–247). Similar to this, CB1R stimulation has been shown to

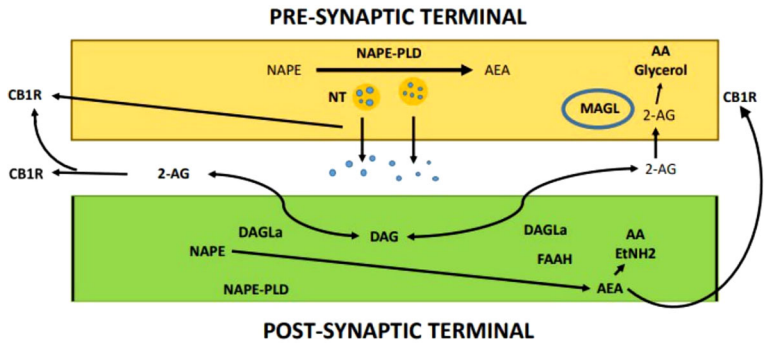

FIGURE 4
Endocannabinoid-mediated synaptic transmission.

activate p38 in rat/mouse hippocampus slices, transfected Chinese hamster ovary (CHO-K1) cells, and human vascular endothelial cells (248). In transfected CHO-K1 cells, JNK activation has been demonstrated, and G proteins, PI3K, and the transduction was mediated by the reticular activating system (Ras) (249). Additionally, JNK initiation was seen in Neuro2A cells that express CB1R endogenously, which may be connected to CB1R-mediated neurite propagation (250).

The CB1R is able to communicate in a G protein-independent manner by linking with additional molecules such as -arrestin, in addition to the conventional G protein-dependent communication present with all G protein coupled receptors (GPCRs) (244). GPCR desensitization is primarily mediated by β -arrestin. β -arrestin attaches to the receptor after GRK phosphorylates it, starting the internalization process, during which β-arrestin may mediate signaling pathways (251). It has been demonstrated that β-arrestin 2-dependent desensitization of the CB1R occurs in a variety of settings (252, 253). According to research done in transfected human embryonic kidney cells (HEK-293), the timing of ERK1/2 phosphorylation in response to CB1R activation is controlled by β-arrestin 2-mediated desensitization but not by CB1R internalization (254). Additionally, follow-up investigations showed a beneficial relationship between the duration of CB1R association with -arrestin at the cell surface in a ligand-specific way and the degree of β-arrestin-mediated signaling (246). Studies with mice deficient in -arrestin 2 have indicated that this protein is crucial for controlling CB1R activity (255, 256). The CB1R expression in the -arrestin 2 knockout mice was similar, but they were more sensitive to THC, with improved antinociception and reduced tolerance (255, 256). In response to the CB1R allosteric modulator ORG27569. A recent study revealed that MAPK kinase ½, ERK1/2, and the proto-oncogene tyrosine-protein kinase Src are all phosphorylated by -arrestin 1, highlighting a signaling mechanism that is heavily reliant on stimuli (257).

In addition to MAPK signaling, the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) pathway also plays a significant role in regulating cell growth and death. The CB1R has been demonstrated to activate the PI3K/Akt pathway in Ratus ratus fundamental astrocytes, the Homo sapiens astrocyte cell line, and transfected CHO-K1 cells, which is in charge of the CB1R-induced protective role on cell survival (245). The PI3K/Akt pathway is used by rat oligodendrocyte progenitors to regulate cell differentiation and improve cell survival against food restriction (258, 259). Similar to this, HU-210, a selective CB1R agonist, protects against the neurotoxin (S)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid in cultured rat cortical neurons by activating the PI3K/Akt pathway but not the MAPK pathways (260). In various brain areas, acute THC treatment in mice activated the PI3K/Akt pathway but not the ERK1/2 pathway (260). Recent research on huntingtin knock-in striatal neuronal cells showed that PI3K/Akt signalling increased the expression of brain-derived neurotrophic factor (BDNF), which allowed CB1R to defend neurons against excitotoxicity (261). Additionally, it has been demonstrated that CB1R-mediated PI3K/Akt activation influences oocyte maturation and embryonic development (262).

Treatment of Peripheral Blood Mononuclear Cells (PBMC) by THC or CBD significantly reduced the mitogen-induced synthesis of neopterin, a cellular immunity marker. However, the pretreatment of PBMC with nanomolar doses of THC or CBD increased the amount of Interferon-gamma (IFN-γ) secreted in response to phytohemagglutinin (PHA), micromolar dosages effectively reduced the amount of this pro-inflammatory cytokine produced as a result of activation (263). Additionally, the biphasic effects of THC and CBD were seen in the mitogen-induced breakdown of the tryptophan, which is mediated by indoleamine-2,3-dioxygenase, and is a crucial adaptive immune defense mechanism (263).

15 Challenges with use of cannabinoids in animals

Major challenges and limitations that may have an impact on the potential use of cannabinoids in animals are:

15.1 Cannabinoid's stability and durability during storage, heating, and exposure to light and oxygen

Stability studies, a vital component of pharmaceutical research, enable the capacity to assess the therapeutic effects of an active pharmaceutical ingredient (API) or a finished pharmaceutical output while taking numerous environmental factors into account. Understanding CBD's physical, chemical, and biological properties as well as information on its stability and shelf life is crucial to guarantee that it is utilized correctly in medicine. While Carbone et al. (264) gave an essentially comprehensive overview of THC degradation products. Cannabis resin and extract were shown to be extremely sensitive to oxygen-induced disintegration, light, and temperature (265). Layton et al. (266) concentrated more on the identification of degradation products generated by the aforementioned conditions, however because of the length of the experiment and the use of methanolic matrices, the results are not totally pharmaceutically acceptable.

It was important to discover an efficient, sensitive, and selective analytical approach for the detection and quantification of CBD and its potential degradation products in order to assess the impact of heat, humidity, oxygen access, matrix, and light. The literature mentions a few studies where cannabinoids were measured in cannabidiol-rich products using a combination of ultra-violent detection coupled with electrospray ionization tandem mass spectrometry (UV and MS/MS), while cannabinoids were analyzed in different matrices using isocratic and gradient elution profiles (267–269). It is challenging to separate cannabinoids under isocratic conditions because of their unique physical and chemical properties (268, 270). The results of the stability study on CBD powder were supported by an experiment on stability that examined how dried cannabis plant material would react to greater temperatures. They demonstrated that heat exposure at

37°C and 50°C results in a considerable loss of cannabinoids in the first 10 weeks, even if the CBD content in all of the stored materials remained largely steady without any evident deterioration for 100 weeks (271).

Because of the possibility for change when a by-product is saved in light exposure, photostability studies, which are necessary to determine CBD's overall light sensitivity, are critical. Cannabinoids are least stable in photons, according to some scientists, although this also depends on other circumstances including the chemicals in which they are saved, temperature, O_2 access, and many other aspects (272). THC and CBD were stable for 6 days when exposed to both naturally occurring and artificial if stored in both crude extract and solution for, indicating that light-exposed samples stored in different solvents degraded quicker than ones held in the dark. The key inference that can be made is that while prolonged exposure to light alone does not significantly alter the CBD concentration, light may hasten the process of degradation when paired with other factors like as the solvent employed, high temperature, and the presence of oxygen.

15.2 Problem in maintaining homogeneity in cannabinoids content in final products

During cannabis extraction operations, specific chemicals and solvents are routinely used, including propane, water, hydrocarbons, ethanol, butane, acetone, isopropanol, and hexane (273, 274). In addition to being employed by illegal extraction operations, these solvents are also used to lower production expenditure and retain terpenes that were already expended (275, 276). According to a current study of fifty-seven cannabis samples, more than 80 percent of the concentrates tested included residual solvents (277). This resulted from the use of chemicals in machine operations and product packaging when processing cannabis (278). Terpenes are being added to tinctures, vape oils, lotions, meals, and beverages by manufacturers of cannabis concentrates and derivative goods to improve flavor, assert health advantages, or recreate the original terpene profile that was lost during the cannabis extraction process. To modify the product's viscosity and reduce production costs, medium-chain triglycerides, propylene glycol, or polyethylene glycol are also added to vape oil (279). Whether they are synthetic, botanical, or cannabis-derived, these additional terpenes represent another potential source of leftover solvents in cannabis-infused products. Additionally, throughout the vaping process, additional terpenes and thinning/cutting agents may collaborate or experience thermoxidative degradation to create, among other things, analytes used in residual solvent compliance testing (280). Although they fall outside the current compliance rules, residual solvents created in this way are nevertheless a significant public health concern. The bulk of published residual solvents test regulations refer to USP 467, the standard for pharmaceutical goods in the industry (280). The testing methodologies defined in USP 467 have been utilized for many years, and the usual solvents encountered in drug components, excipients, and final products were clearly recognized.

Analytical technologies are established to know potential of *Cannabis sativa* and its derivative products as pollutants. However, there would be a continual urge to develop the universal methodology to cannabis testing as more testing data are gathered, more proficiency testing programs are assessed. This would continue to support consumer safety and lead the development of laws and testing standards for goods derived from hemp. Consider potency as an illustration; it continues to be a key factor in the cannabis industry's widespread consumer preference (280). Testing labs and their support services will continue to face challenges as the market for cannabis derivatives develops due to tighter regulatory oversight and an increase in the variety of matrices. Additionally, as other cannabinoids, such as 8-THC, come under regulatory oversight, new laboratory tools may be required to satisfy method specificity criteria. For the other test techniques mentioned in this article, comparable sets of difficulties exist (280).

It is also important to remember that secondary metabolites of interest in cannabis go beyond terpenes and cannabinoids. Flavonoids are one of several additional compounds of interest that could be used in cannabis testing (281). When these criteria become reality, the analytical testing community will need to use what it has learned about cannabis' difficulties as a matrix to build appropriate testing procedures. Fortunately, significant advancements have lately been achieved in our comprehension of the constraints placed on analytical testing of cannabis and its byproducts. This is a direct result of regulatory changes that have allowed cannabis science to enter the commercial market. When there are monetary benefits, there will be increase in effective testing regimes, though not beyond increasing pains because of delay in the accessibility of the crucial testing framework (280).

15.3 Lack of global standardized regulation on the use of hemp and cannabinoids

There are many CBD products available, some of which are marketed as medicines for various conditions as well as other items that are produced and disseminated without regulations and frequently have unproven ingredients (282). The U.S. Food and Drug Administration has sent manufacturers 2 major series of caution notifications for false medical assertions (explaining health assistance and wellbeing without any supporting data) and false production claims (marketing products as having a certain concentration of CBD when testing shows that it doesn't (282).

16 Prospects of using cannabinoids as potential feed additive in animals

The hemp plant can be used to produce a variety of feed materials, including hemp seed meal/cake, *Cannabis sativa* seed oil, and the entire herb (including *Cannabis sativa* seed shives, fresh or dried). *Cannabis sativa* flour (ground dried *Cannabis sativa* leaves) and *Cannabis sativa* protein segregates (from seeds) are

other goods. All animal species could utilize hemp seed and hemp seed cake as feed, and the EFSA set up most incorporation values in the whole feed for each species, such as 3 to 7 percent for chickens, 2 to 5 percent for *Sus domesticus*, 5 percent for cattle, and 5 percent for aquatic species for hemp *Cannabis sativa*. Additionally, feed conversion ratio studies show that hemp and its derivatives can be used as a suitable supply of vital lipids and crude protein for cattle diets (283).

Due to its high fiber content, the entire hemp plant, including the stem and leaves, is regarded as an acceptable source of food for ruminants (and horses). All species of animals can be fed on *Cannabis sativa* seed and *Cannabis sativa* seed cake. When introducing such goods into the total feed, a number of particular species constraints (fiber for hens, FA for *Sus domesticus*, etc.) should take into account. Hemp seed contains a part of rumen-indigestible protein, which is favorable for ruminants (97). According to information from feeding trials, hemp seed cake might be utilized up to 20 percent in the diets of laying hens; it is therefore determined that no more than 10 percent can be used in the diets of hens for weight gain. Although there is null information on pigs, it is anticipated that 10 percent *Cannabis sativa* seed cake and 5 percent *Cannabis sativa* seed could be utilized in pig complete feed. According to data, dairy cows can get a total mixed ration that contains 14 percent hemp seed cake. Comparable research on the upbringing of calves and fattening of cattle revealed that one to 1.4 kg of *Cannabis sativa* seed cake could be given per day (97).

Because hemp products are extremely limited in terms of quantity and price, the maximum integration rates in the formulation of compound feeding stuffs are probably lower than the aforementioned values; as a result, it is difficult to determine what they would be (47). The following maximal assimilation values in feed could be accepted in normal manufacturing and production if considerable volumes of hemp products are locally accessible: Pigs 2 to 5 percent hemp seed/hemp seed cake; ruminants' 5 percent in the routine daily feed; fish 5 percent; poultry for increase in weight 3 percent and laying poultry 5 to 7 percent. It must be highlighted that these values or numbers cannot be viewed as cumulative as the concurrent application of hemp by products would vastly outweigh available resources. Entire herb (or portions of it, like leaves) may be eaten as forage by ruminant.

17 Conclusion and future prospects

The present study concluded that hemp or its cannabinoids possess excellent potential to modulate health and performance of animals. The active cannabinoids have shown excellent antioxidant and immune-modulatory activities making them promising dietary additives especially under oxidative stress and disease conditions, respectively. Besides the leaves and seeds of Cannabis sativus, its by-products (oil cakes etc.) also are being used in animal feeds as supplements. Different treatment strategies (e.g ensiling or solid-state fermentation) have been used to avoid some adverse effects of Cannabis feeding on animals. However, further studies are required to optimize best feeding levels of hemp and cannabidiols in animal diets to get desirable outputs in terms of better health and production of animals. Moreover, in-depth research will be needed to understand the therapeutic efficacy of cannabinoids on various health aspects in diverse animal species, examining optimal dosage and administration methods, exploring potential side effects and safety profiles, and delving into the underlying mechanisms of cannabinoid action. Additionally, long-term impacts and feasibility of incorporating cannabinoids into veterinary practices could be the crucial aspects for future research.

Author contributions

F-UH: Conceptualization, Writing – original draft. CL: Software, Writing – review & editing. MM: Writing – review & editing. RB: Writing – review & editing, Data curation, Visualization. MA: Writing – review & editing, Validation. FS: Writing – review & editing. FC: Writing – review & editing, Investigation, Methodology. YL: Writing – review & editing, Formal Analysis. JZ: Writing – review & editing, Software, Validation. PS: Writing – review & editing, Formal Analysis. BL: Writing – review & editing, Investigation, Methodology. QL: Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Enhancing bovine immune, antioxidant and anti-inflammatory responses with vitamins, rumen-protected amino acids, and trace minerals to prevent periparturient mastitis

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Mastitis, the inflammatory condition of mammary glands, has been closely associated with immune suppression and imbalances between antioxidants and free radicals in cattle. During the periparturient period, dairy cows experience negative energy balance (NEB) due to metabolic stress, leading to elevated oxidative stress and compromised immunity. The resulting abnormal regulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), along with increased non-esterified fatty acids (NEFA) and β-hydroxybutyric acid (BHBA) are the key factors associated with suppressed immunity thereby increases susceptibility of dairy cattle to infections, including mastitis. Metabolic diseases such as ketosis and hypocalcemia indirectly contribute to mastitis vulnerability, exacerbated by compromised immune function and exposure to physical injuries. Oxidative stress, arising from disrupted balance between ROS generation and antioxidant availability during pregnancy and calving, further contributes to mastitis susceptibility. Metabolic stress, marked by excessive lipid mobilization, exacerbates immune depression and oxidative stress. These factors collectively compromise animal health, productive efficiency, and udder health during periparturient phases. Numerous studies have investigated nutrition-based strategies to counter these challenges. Specifically, amino acids, trace minerals, and vitamins have emerged as crucial contributors to udder health. This review comprehensively examines their roles in promoting udder health during the periparturient phase. Trace minerals like copper, selenium, and calcium, as well as vitamins;

have demonstrated significant impacts on immune regulation and antioxidant defense. Vitamin B12 and vitamin E have shown promise in improving metabolic function and reducing oxidative stress followed by enhanced immunity. Additionally, amino acids play a pivotal role in maintaining cellular oxidative balance through their involvement in vital biosynthesis pathways. In conclusion, addressing periparturient mastitis requires a holistic understanding of the interplay between metabolic stress, immune regulation, and oxidative balance. The supplementation of essential amino acids, trace minerals, and vitamins emerges as a promising avenue to enhance udder health and overall productivity during this critical phase. This comprehensive review underscores the potential of nutritional interventions in mitigating periparturient bovine mastitis and lays the foundation for future research in this domain.

KEYWORDS

periparturient period, mastitis, dairy cattle, immunity, antioxidant status, antiinflammation, amino acids, trace minerals

1 Introduction

Mastitis, characterized by the inflammation of mammary glands (1, 2), is intricately associated with immune suppression and an imbalance between free radicals and antioxidants within the animal's physiological framework (3–5). Dairy cows confront the challenge of negative energy balance (NEB) during the periparturient period, precipitating a cascade of detrimental effects such as reduced dry intake, metabolic stress, hormonal fluctuations, heightened oxidative stress, and compromised immune responses (6, 7).

This NEB triggers the mobilization of fat reserves, culminating in the dysregulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (8), along with elevated levels of non-esterified fatty acids (NEFA) (9, 10) and β-hydroxybutyric acid (BHBA) (11). The notable concentrations of NEFA and BHB compromise the bovine immune system, rendering dairy cattle more susceptible to infections (12). Scientific investigations have demonstrated that heightened NEFA and BHBA levels exert inhibitory effects on bovine peripheral blood mononuclear cells (BPMCs) (13), stifle interferon production (14), and impede the functionality of polymorphonuclear neutrophils (PMNLs) (15). Furthermore, escalated levels of NEFA and BHB serve as indicators of mastitis susceptibility (16, 17). During the periparturient period, the heightened concentrations of NEFA and BHB emerge as pivotal factors undermining immune function, directly augmenting the vulnerability to mastitis (11).

In addition to NEB-related factors, other metabolic disorders like ketosis and hypocalcemia indirectly contribute to mastitis in dairy cattle (18–20). Hypocalcemia prompts cows to spend more time lying down, resulting in teat exposure to physical injury and facilitating pathogen entry. The compromised sphincter muscle integrity of teats and diminished immune functionality due to reduced calcium levels emerge as pivotal factors linking mastitis with hypocalcemia.

In the realm of normal physiological conditions, the intricate antioxidant system adeptly mitigates and eradicates ROS stemming from metabolic processes. Nevertheless, the transitions accompanying pregnancy and calving instigate an overabundance of ROS production (21, 22). This disruption in the equilibrium between ROS generation and the availability of antioxidants ushers in oxidative stress, rendering cattle more susceptible to a spectrum of maladies (23, 24). The unrestrained ROS production culminates in lipid peroxidation, tissue impairment, and fluctuations in reduced glutathione (GSH) levels, a pivotal constituent of glutathione metabolism (24, 25). Oxidative stress inflicts damage upon the structure and function of cellular macromolecules, including lipids, proteins, and nucleic acids, thereby inciting metabolic dysfunctions and ailments, notably mastitis in dairy cattle (6, 26). Sustaining redox homeostasis during the periparturient and peak lactation phases is of paramount importance (27–29). Oxidative stress associated with parturition may contribute to immunological and inflammatory aberrations, heightening the vulnerability to metabolic and infectious disorders (22, 30).

The metabolic stress encountered during the periparturient period stands as another pivotal factor exposing animals to immune suppression and the deviant regulation of oxidative stress. This phase instigates excessive lipid mobilization, subsequently leading to oxidative stress (16). The abnormal regulation of immunity and inflammation driven by metabolic and oxidative stress constitutes the third critical factor predisposing dairy cattle to periparturient mastitis (3, 31–33). Furthermore, oxidative and metabolic stress, negative energy

balance, immune suppression, and diminished productive efficiency collectively undermine the overall productivity of these animals (34–36).

Numerous nutritional strategies have been employed to address the challenges encountered during the periparturient period in dairy cattle (37–39). Extensive research endeavors have been undertaken to probe the intricate interplay between nutrition, immune modulation, and the augmentation of antioxidant status, thereby fostering enhanced udder health during this critical phase (40–45). O'Rourke (41), in particular, highlighted that cows undergoing negative energy balance face an elevated risk of ketosis, with clinical ketosis being associated with a twofold increase in the likelihood of clinical mastitis. Furthermore, extant research underscores the pivotal role of nutritional disturbances during the periparturient period in contributing significantly to immune suppression, oxidative stress, and metabolic perturbations (46–48).

Among the pivotal nutrients that have garnered attention, specific amino acids, trace minerals, and vitamins have exhibited a pronounced impact on udder health during the periparturient period, particularly in the prevention of mastitis in dairy cattle. Consequently, this review aims to provide a comprehensive exploration of the contributions made by trace minerals, vitamins, and amino acids in bolstering udder health during the critical periparturient phase in dairy cattle.

2 Key factors associated with periparturient bovine mastitis

2.1 Metabolic stress, NEFA and BHBA

The NEFA and BHBA represent metabolites resulting from the mobilization of fat reserves triggered by NEB during the perinatal period in dairy cows, exerting detrimental effects on the cellular physiology of various bovine cell types (12). Severe NEB initiates lipid mobilization, leading to elevated circulating concentrations of NEFA and BHBA. Clinical data derived from previous studies have established a strong correlation between heightened levels of NEFA and BHBA and an increased incidence of postpartum diseases, including mastitis, ketosis, clinical endometritis, metritis, and other conditions associated with immunosuppression. These conditions have adverse repercussions on the overall health, longevity, as well as the productive and reproductive performances of dairy cows (11, 49).

Furthermore, a study conducted by Li et al. revealed that NEFA and BHBA lead to increased accumulation of malondialdehyde (MDA) and ROS, along with reduced total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px) activity, resulting in oxidative stress. Additionally, NEFA and BHBA stimulation led to heightened expression of inflammatory markers such as nitric oxide (NO), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-1 beta (IL-1β). Mechanistically, their data demonstrated that NEFA and BHBA activate the mitogen-activated protein kinase (MAPK) signaling pathway, shedding light on the fact that NEFA and BHBA induce oxidative stress and an inflammatory response, potentially via the MAPK signaling pathway in BMECs (12). Similarly, another study observed that elevated NEFA levels increased ROS levels, thereby activating the MAPK signaling pathway and triggering ER stress-mediated apoptosis in BMECs (50).

2.2 Oxidative stress

Oxidative stress induced by metabolic stress is closely associated with several pathological conditions, including mastitis, during the periparturient period in dairy cattle (26, 51, 52). Consistently, Sordillo and Aitken (3) reported that oxidative stress resulting from negative energy balance is intricately linked with impaired immunity, subsequently leading to heightened inflammation, thereby rendering dairy cattle more susceptible to mastitis (3, 53; 54). In a similar vein, a study noted that selenium supplementation effectively enhanced the total antioxidant capacity of dairy cattle, consequently mitigating the risk of mastitis in periparturient dairy cattle (55). Additionally, when BMECs were exposed to lipopolysaccharide (LPS), it was observed that ROS levels increased, accompanied by inflammatory changes (56, 57). In Table 1, we present a comprehensive summary of recent published research findings that elucidate the intricate relationship between oxidative stress and mastitis in dairy cattle during the critical periparturient period. The interplay among multiple factors, including metabolic stress/oxidative stress, immunity, inflammation, NEB, and susceptibility to mastitis, has been depicted and summarized in Figure 1.

TABLE 1 Recent studies reported association of metabolic disturbances, suppressed immunity and oxidative stress with periparturient bovine mastitis.

S.No	Outcomes of oxidative stress	Impact on udder health of dairy cattle	References
1	Impaired immune response and abnormal regulation of inflammation by metabolic induced oxidative stress	Susceptible to mastitis	5, 36, 56, 58
2	Inhibition of nuclear factor erythroid 2 related factor 2 (NFE2L2), a master regulator of cellular redox homeostasis was followed by decreased level of GSH-Px, catalase (CAT), and superoxide dismutase (SOD) and increased level of ROS and MDA in response to exogenous free fatty acids (FFA) in bovine mammary epithelial cells	Induced inflammatory changes in mammary gland	59

(Continued)

TABLE 1 Continued

S.No	Outcomes of oxidative stress	Impact on udder health of dairy cattle	References
3	Increased levels of ROS, oxidative stress index (OSi), and decreased α-tocopherol (α-T) and serum antioxidant capacity (SAC) levels around parturition	At high risk of developing mastitis	60
4	The NEFA and BHBA induced oxidative stress and suppressed immunity in BMECs	Increased chances of mastitis	12, 50
5	Low level of GSH, SOD, CAT, and total antioxidant capacity (T-AOC); higher levels of ROS and MDA	Associated with mammary gland inflammation	61
5	Elevated oxidative stress	Increased the susceptibility of dairy cattle to mastitis	62
6	The progressive development of oxidative stress during the transition from late gestation to peak lactation is thought to be a significant underlying factor leading to dysfunctional immune cell responses.	Expose dairy cattle susceptibility to infections including mastitis	55
7	Elevated level of serum amyloid A (SAA), MDA and decreased level of total antioxidant capacity	Increased risk of mastitis	63

3 Trace minerals role in bovine mastitis prevention during periparturient period

3.1 Reference values for serum trace minerals in dairy cows

To establish appropriate dietary recommendations, it is essential to consider reference values for serum trace mineral concentrations in dairy cows. Notable values include: calcium (2.2–2.6 mmol/L) (64), phosphorus (1.3–2.6 mmol/L) (64), magnesium (0.75–1.0 mmol/L) (65), selenium (0.73–1.08 µmol/L) (66), copper (1–18 µmol/L) (67), and zinc (8–19 µmol/L) (68). Meeting these reference values can help optimize the mineral supply and overall health of dairy cattle.

3.2 Role of trace minerals in health regulations of dairy cattle

In the field of bovine veterinary medicine, the pivotal role of mineral deficiencies in modulating the immune system should not be underestimated. Recent investigations have elucidated that the provision of micronutrient supplements has demonstrably reduced the count of milk somatic cells (SCC), bolstered immune function, and elicited anti-inflammatory and antioxidant responses in dairy cattle during the periparturient period (69, 70). Extensive research has shown that these deficiencies can lead to immunosuppression, making the animals more susceptible to infectious diseases such as mastitis (42). Consequently, addressing mineral imbalances in cattle health becomes crucial in maintaining optimal health outcomes. Minerals typically constitute essential structural components within the body, acting as crucial cofactors for diverse enzymes and participating in vital processes such as nerve signaling, muscle contraction, and the regulation of proper keratosis. Insufficient mineral levels can result in diminished immune cell activity or disruption of innate defense mechanisms within the breast, thereby fostering the progression of mastitis (71). Recent studies have shown promising results regarding the supplementation of trace minerals and its effect on dairy cattle health by enhancing their immune and antioxidant status during the periparturient period (72–74). Trace mineral supplementation has been found to significantly enhance immune and antioxidant status, contributing to reduced levels of NEFA (75) and alleviating inflammatory changes, which are critical factors associated with clinical mastitis (76–80). These studies emphasize the potential of mineral supplementation as a strategy to improve cattle health and overall herd performance. Minerals play a fundamental role in maintaining the immune system of cattle. Recent studies have highlighted the association between serum trace mineral concentrations and their impact on bovine health. For instance, higher concentrations of serum selenium and phosphorus have been linked to the successful cure of bovine clinical mastitis (81). Similarly, research demonstrated that subcutaneous supplementation of specific minerals, such as zinc, manganese, selenium, and copper, led to increased SOD activity, decreased serum BHBA concentrations, reduced milk SCC, and a lower incidence of mastitis (82). These findings underline the vital role of trace minerals in preventing and mitigating the effects of mastitis in dairy cattle.

In clinical practice, certain mastitis cases may require supportive therapy, including the administration of calcium-containing fluids, due to the prevalent occurrence of hypocalcemia in cows with udder inflammation (83). Additionally, the supplementary use of injectable trace minerals, such as zinc, manganese, selenium, and copper, has been considered as an adjunctive tool in mastitis therapy. Clinical evidence suggests that supportive therapy involving the administration of fluids enriched with calcium can be crucial in managing mastitis in dairy cows (83). Hypocalcemia, commonly observed in cows with udder inflammation, can compromise immune function and hindering the healing process. Proper calcium supplementation has shown promise in improving the overall therapeutic outcomes in mastitis cases. Recent studies have investigated the use of injectable trace minerals as a complementary approach in the treatment of mastitis in dairy cows. Hoque et al. (84) emphasized the significance of antimicrobial therapy as the primary treatment for mastitis. However, their experiment revealed a noteworthy finding – cows that received only selenium preparations

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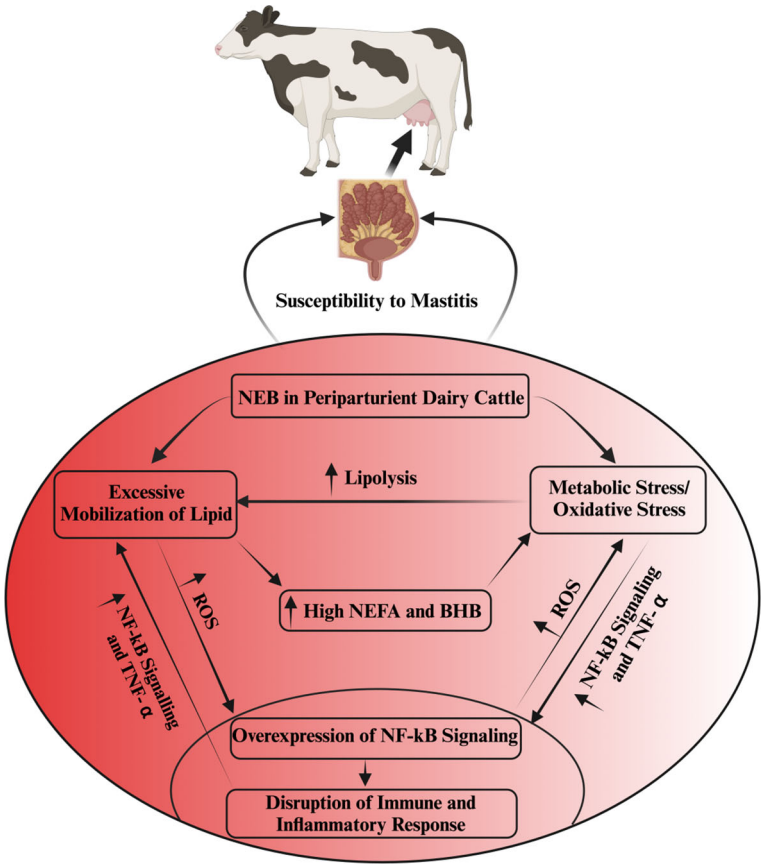

FIGURE 1
The interlink among metabolic stress, immunity, inflammation and mastitis susceptibility. Oxidative stress disrupts immune and inflammatory functions via the activation of NF-kB signaling. This aberrant inflammatory regulation, in turn, fosters excessive TNF-α production in non-phagocytic cells, resulting in heightened oxidative stress and increased lipolysis. The intricate interplay of oxidative stress, escalated lipid mobilization, and compromised immune and inflammatory processes is predominantly associated with NEB in periparturient dairy cattle. NEB, by triggering an excessive lipid mobilization in these cattle, elevates NEFAs, BHB and ROS, consequently inducing oxidative stress. This oxidative stress further exacerbates the disruption of immunity and inflammation regulation, rendering dairy cattle more susceptible to mastitis.

demonstrated reduced susceptibility to udder inflammation compared to the untreated control group. Ganda et al. (81) conducted a study involving the injection of trace minerals, including zinc, manganese, selenium, and copper, in dairy cows with mastitis. The results indicated a reduction in the number of chronic mastitis cases, showcasing the potential of injectable trace minerals in mitigating the severity and persistence of the disease.

Another noteworthy study by Machado et al. (85) explored the effects of injecting a multimineral preparation containing selenium, copper, zinc, and manganese. Their findings demonstrated a positive impact on udder health, leading to a decrease in linear SCC, and a reduction in the incidence of subclinical and clinical mastitis cases. Furthermore, the researchers reported an increase in serum SOD activity, indicating potential antioxidant benefits without affecting leukocyte function (82). Despite the positive effects observed in other studies, Ferreira and Petzer (86) reported no significant correlation between SCC and the levels of selenium in milk or serum among cows supplemented with selenium in various forms. This suggests that other factors might influence the relationship between SCC and selenium supplementation. A separate investigation by Bourne et al. (87) investigated the supplementation of vitamin E with selenium. The study revealed a notable 10% reduction in the risk of culling and mastitis rate, suggesting a potential synergistic effect between vitamin E and selenium in enhancing mastitis treatment outcomes. Recent research conducted by Smulski et al. (88) indicated that combining an antibiotic treatment with an antioxidant containing selenium slightly improves the overall effectiveness of clinical mastitis treatment. This highlights the importance of considering combination therapies to optimize mastitis management in dairy cows. Further investigation is necessary to gain a comprehensive understanding of its effects on cattle health and immune responses. Figure 2 illustrates the pivotal role of trace mineral supplementation in health regulation, encompassing its capacity to enhance immune function, mitigate inflammation, and bolster antioxidant responses among periparturient dairy cattle.

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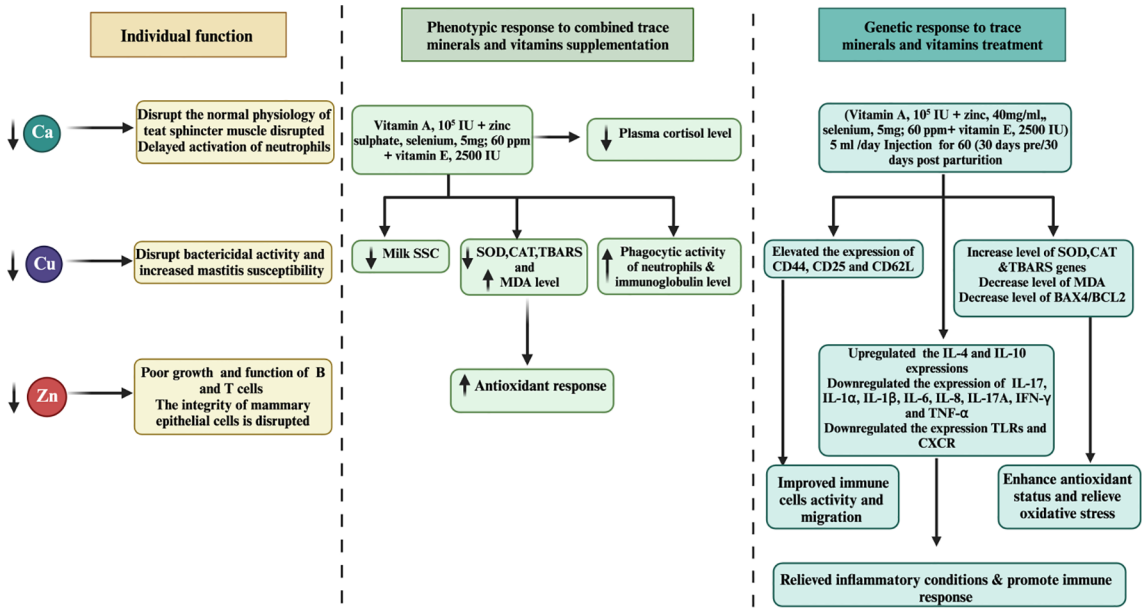

FIGURE 2
Associations between molecular and phenotypic factors related to immunity, apoptosis, milk SCC, anti-inflammatory responses, and antioxidant activity in dairy cattle following combined treatments with minerals and vitamins are examined. Over a 60-day duration, the combined treatment of minerals and vitamins exhibited notable downregulation of apoptosis and inflammation-related gene expression levels, concomitant with upregulation of genes associated with immunity, antioxidation, and pro-inflammatory responses in periparturient dairy cattle. Furthermore, the integrated regimen of trace minerals and vitamins demonstrated enhancements in neutrophil phagocytic activity, accompanied by reductions in plasma cortisol and milk SCC levels, suggesting a potential association with mastitis prevention during the periparturient period in dairy cattle.

3.3 Specific

3.3.1 Role of calcium, copper, zinc, and selenium in regulating anti-inflammatory and immune responses to prevent mastitis

Calcium assumes a pivotal physiological role within the biology of living organisms, functioning as an indispensable constituent of bodily structures and a critical determinant for muscle contractions, encompassing both skeletal and smooth muscles, such as the mammary gland sphincter. A prominent periparturient metabolic affliction, known as hypocalcemia, arises due to the excessive depletion of calcium in colostrum and milk. Consequently, the requisite calcium level is constrained to remain below 1.5 mmol/L to maintain normal muscle physiology, resulting in afflicted animals assuming a protracted recumbent posture (89). Furthermore, diminished calcium levels also correlate with compromised phagocytic activity of neutrophils and suppressed immunity, predisposing dairy cattle to other periparturient diseases (90, 91). In addition, the incapacity of the mammary gland sphincter muscle to contract leads to prolonged teat opening, creating favorable conditions for pathogenic microorganisms to induce mastitis (92). Notably, a study revealed lower calcium levels in acute *Escherichia coli (E.coli)* mastitic cows compared to healthy counterparts (93). Moreover, investigations have unveiled that certain polymorphisms (G519663A and G38819398A) within the Calcium channel, voltage-dependent, alpha-2/delta subunit 1 (CACNA2D1) gene are associated with mastitis resistance in Sahiwal cattle (94, 95).

Copper is another trace mineral, plays a crucial role in the structure and catalytic properties of cuproenzymes, which are enzymes that incorporate copper (96). Notable examples of cuproenzymes include cytochrome-c oxidase, superoxide dismutase, catechol oxidase, ceruloplasmin, and amine oxidases. Additionally, copper holds a significant position among enzymes, ranking as the second most prevalent metal after zinc and contributing to their proper functioning (97). Apart from its involvement in enzymatic functions, copper also plays a vital role in various physiological processes. It contributes to essential processes such as collagen and elastin synthesis, which are crucial for tissue structure and elasticity. Furthermore, copper is involved in myelination, a process critical for nerve impulse conduction, and hemoglobin production, which is essential for oxygen transport (96). Copper has been recognized for its antibacterial properties against bacteria commonly isolated from mastitic cows. Research by Reyes-Jara et al. (98) highlights that even low copper concentrations, as low as 250 ppm, can effectively inhibit the growth of mastitis microorganisms like *E.coli* and coagulase-negative *Staphylococci*. Supporting studies by Wernicki et al. (99) and Kalińska et al. (100) demonstrate the potent antimicrobial activity of silver and copper nanoparticles against bacteria derived from inflamed udders, indicating copper preparations as potential alternatives to dipping solutions. *In vivo* investigations have revealed promising results concerning the effects of copper supplementation. A 100-day dietary copper supplementation study, as conducted by Scaletti et al. (101),

showed a reduced clinical response in Holstein cows experimentally intramammary infected with *E. coli*. The experimental group, supplemented with copper at a concentration of 20 ppm, exhibited improved outcomes compared to the control group with 6.5 ppm copper concentration. Likewise, Gakhar et al. (102) observed a decreased incidence of postpartum mastitis in cows supplemented with copper, further validating the potential benefits of copper supplementation. Copper deficiencies have been associated with impaired phagocytosis and decreased Cu, Zn-SOD (copper-zinc superoxide dismutase) activity. These findings underscore the importance of adequate copper levels for a properly functioning immune system (103). The antibacterial properties of copper are explained by the oxidation-mediated disruption of bacterial lipids, proteins, and DNA. This mechanism highlights the potential of copper as an effective antimicrobial agent (104).

Zinc is a vital trace element that plays a pivotal role in the maintenance of rumen microbiota and the synthesis of essential proteins, including collagen, glucagon, insulin, DNA, and RNA (105). It serves as an indispensable activator for a diverse array of enzymes, encompassing alkaline phosphatase, carbonic anhydrase, DNA and RNA polymerase, and, in conjunction with copper, superoxide dismutase, which assumes a key role in antioxidant processes (97). Furthermore, zinc acts as a crucial co-factor for a series of oxidoreductases and significantly contributes to keratin formation. Studies have shown that dietary supplementation of zinc can have a significant impact on the health of dairy cattle. Specifically, some research has reported that zinc supplementation results in reduced somatic cell count (106, 107) and decreased milk amyloid A levels (106). However, contrasting findings were observed by Whitaker et al. (108), where no effect of dietary zinc supplementation on SCC was found. The integrity of the intact mammary epithelium, which acts as an impermeable barrier to microorganisms, is recognized as an intrinsic component of the udder immune system. Notably, studies have demonstrated that the supplementation of zinc preparations in Holstein cows can lead to improved integrity of the mammary epithelium (109), although contradictory results have been reported by Shaffer et al. (110). Zinc is crucial for the development and proper functioning of cells involved in innate immunity, such as neutrophils. Deficiency of this essential mineral adversely affects the growth and function of T and B cells, impacting the overall immune response in dairy cattle. Zinc exhibits potent antioxidant properties and plays a pivotal role in stabilizing cellular membranes. This feature suggests its significance in preventing free-radical-induced injuries during inflammatory processes (111).While the supplementation of trace minerals has demonstrated the capacity to augment immune responses and ameliorate inflammatory alterations, it is imperative to emphasize the need for extensive and in-depth investigations aimed at elucidating the precise underlying mechanisms and exploring the potential therapeutic applications of such supplementation in the context of mitigating mastitis in dairy cattle.

3.3.2 Role of selenium of in regulation of inflammatory and immune response to prevent mastitis

Selenium has garnered considerable attention as a crucial element with antioxidant and immune-regulatory properties (36). Extensive research, encompassing *in-vitro* and *in-vivo* studies, has demonstrated that Se supplementation can alleviate inflammatory changes, oxidative stress, and mastitis caused by *S. aureus* in mice mammary glands (112–114). Plasma GPx-3, an extracellular antioxidant protein containing selenocysteine, plays a vital role in reducing hydrogen peroxide and lipid hydroperoxides (115). GPx-3 is essential for the antioxidant defense mechanism in dairy cattle (116). Furthermore, milk lactoserum obtained from selenium-fed cows has shown undefined antibacterial action, potentially attributed to the elevated level of GSH-Px resulting from selenium treatment (117–119). Selenium supplementation has also been found to regulate antioxidant-associated genes (SOD, GPX, TOAX, GSH, and CAT) and suppress oxidative stress in dairy cattle, thus preventing oxidative stress and subsequent infections (120). The pivotal role of selenium in regulating immunity and antioxidant activity has made it a primary focus in mastitis control research for dairy cattle. Studies have indicated a positive correlation between low levels of selenium and GPx with oxidative stress in periparturient dairy cattle. However, desired selenium supplementation in mammary epithelial cells has been found to promote anti-oxidative responses, leading to a reduction in apoptotic cells (121). Moreover, low GPx levels in blood were associated with a higher percentage of mammary infections (mastitis) in periparturient dairy cows (122). Recent research indicates that S. aureus has the ability to modulate myeloid differentiation factor 88 (MYD88) and engage the NF-kB signaling pathway, thereby initiating inflammatory responses within the mammary gland through its interaction with toll-like receptor 2 (TLR-2) (123, 124). In a recent investigation by Wei et al. (125), an elevation in MYD88, IL-1β, TNF-α, pyrin domain-containing protein 3 (NLRP), caspase-recruitment domain (ASC), and caspase-1 levels was observed in *S. aureus*-infected macrophages in mice. Remarkably, a 90-day regimen of selenium treatment led to a significant reduction in the expression of MYD88, IL-6, IL-1β, NLRP3, and ASC within the macrophages (125).

During instances of microbial infection and cellular damage, the NLRP3 inflammasome—a vital component of the innate immune system—maintains regulation over caspase-1 activation and the secretion of pro-inflammatory cytokines such as IL-1β/IL-18. However, *S. aureus*-mediated irregular control of the NLRP3 inflammasome within the mammary gland leads to an atypical inflammatory response (126, 127). Supplementing with selenium was shown to significantly curtail the expression of NLRP3 inflammasome and proinflammatory cytokines IL-1β/IL-18, thereby mitigating the abnormal inflammatory response and consequently preventing mastitis in mice (112, 127). Furthermore, Se supplementation effectively restrained the nuclear transcription factor-kappa B (NF-κB) and MAPK signaling pathways that are intricately involved in the progression of mastitis within murine macrophages (128, 129). This suggests that selenium

supplementation holds potential in suppressing the inflammatory alterations elicited by *S. aureus*.

NF-κB and MAPK signaling pathways wield pivotal roles in initiating inflammatory transformations by bolstering cytokine production during *S. aureus*-induced mastitis in the mammary gland (113, 130, 131). This observation is consistently upheld by Liu et al. (113), who found that LPS supplementation led to a reduction in the recruitment of neutrophils and macrophages in mammary epithelial cells. Moreover, the overexpression of TLR2, IL-1β, TNF-α, and IL-6, coupled with the heightened phosphorylation of NF-κB and MAPKs proteins resulting from *S. aureus* exposure, were markedly alleviated through Se supplementation in mouse models (92, 112, 113, 132).

MerTK has been extensively documented as a key player in regulating the PI3K/AKT/mTOR pathway to enhance anti-inflammatory capabilities. Activation of the PI3K/Akt pathway within macrophages by MerTK can effectively impede NF-κB signaling (133). Furthermore, MerTK-mediated modulation of the PI3K/AKT/mTOR pathway exhibited repressive effects on TLR2-triggered immune responses, resulting in diminished inflammatory reactions and oxidative stress in U937 cells (134). *S. aureus* in its induction of an inflammatory response within the mammary gland of mice increased the levels of IL-1β, IL-6, and TNF-α. Notably, treatment with S. aureus led to decreased phosphorylation levels of MerTK, PI3K, AKT, and mTOR in the mouse mammary gland (114). Conversely, selenium treatment enhanced the phosphorylation levels of MerTK, PI3K, AKT, and mTOR while concurrently reducing the expression of inflammatory cytokines (IL-1β, IL-6, and TNF-α). These observations underscore selenium's potential to augment immunity, enhance antioxidant status, and mitigate the inflammatory response within mammary glands, thereby alleviating mastitis in mice (114). In a recent *in vitro* experimental trail utilizing mammary alveolar cell large T antigen (MAC-T), Jing et al. (135) substantiated that selenium treatment had a marked downregulatory effect on genes (IL1B, IRAK4, MYD88, and SOCS3) linked to mastitis progression in dairy cattle. Furthermore, selenium treatment exhibited inhibitory effects on PI3K/AKT, MAPK, and NF-κB signaling pathways, while concurrently promoting the anti-inflammatory milieu through acceleration of the PI3K/Akt/mTOR pathway in dairy cattle (135). Furthermore, documented evidence indicates that Se can enhance the expression of IL10 and peroxisome-proliferator-activated receptor gamma (PPAR-γ) activity, while concurrently suppressing NF-κB and NO within the mammary gland. These effects hold true for cases of *S. aureus*-induced mastitis (136, 137). Additionally, selenium has been shown to alleviate the oxidative stress and inflammatory state of the mammary gland—a crucial factor in reducing the susceptibility of mice to mastitis (136; Zhang W et al., 2016). Various investigations have demonstrated positive correlations between selenium intervention and notable outcomes, including diminished SCC in milk, elevated levels of GSH-Px and reduced concentrations of MDA in plasma (138–144). Higher MDA levels are directly associated with oxidative stress, which heightens the susceptibility of periparturient dairy cattle to mastitis. Within the realm of animals, a group of twenty-five selenoproteins, twelve of which exhibit antioxidant and immunological functions, have garnered attention as promising candidates for mitigating mastitis in transition dairy cattle (135, 145, 146). The selenium content appears to be a determining factor influencing the vulnerability of mammary glands to bacterial infections during the periparturient period in dairy cattle (122). Additionally, a notable observation was made regarding selenium treatment's efficacy in reducing SCC levels and IL-6 concentrations, while concurrently augmenting GSH-Px activity in dairy cattle (122). The potential of selenium treatment in impeding the growth of mastitis-causing bacteria, such as *Streptococcus uberis (S. uberis), S. aureus, E. coli*, and *Streptococcus agalactiae*, has been documented (147). This approach has also been associated with pronounced antibacterial effects, heightened antioxidant capacity, elevated GSH-Px activity, and lowered SCC levels in the milk of perinatal dairy cows (147). Likewise, other investigations have reported that selenium intervention, by enhancing GSH-Px activity, reduces the likelihood of mammary gland infections in dairy cattle during the transition phase (148, 149). Similarly, a distinct finding was noted, where selenium supplementation outperformed antibiotic treatment during the periparturient period in dairy cattle. To elaborate, among 36 cows, 14 still encountered infections even after antibiotic treatment, while merely 4 out of 36 cows developed mastitis in response to 4 mg of selenium supplementation during the transition phase in dairy cattle (150). Additionally, records indicate that selenium intake enhanced antioxidant capacity and concurrently regulated both innate and adaptive immunity within the mammary glands, effectively countering mastitis in dairy cattle (117). The summary of the studies discussing the association of selenium with health-enhancing phenotypic traits (antioxidant, anti-inflammation, and immunity) has been presented in Table 2. In addition, the mechanism through which selenium prevents mastitis has been highlighted in Figure 3.

4 Exploring the potential of mineral nanoparticles for mastitis mitigation

In animal production, minerals can be integrated into the diet or utilized in therapy through various forms, such as inorganic salts, organic forms, chelates, or nanoparticles (NPs). Nanoparticles have been studied extensively for their potential as animal growth promoters, antimicrobials, and alternatives to conventional cleaning agents (154). A significant advantage of NPs is their ability to avoid bacterial resistance by exerting toxic effects on via DNA degradation, and lipid and protein peroxidation (155, 156). Consistently, recent studies extensively described the role of different types of nanoparticles in the treatment of mastitis with positive results (157, 158). In the literature, various types of minerals nanoparticles containing copper (CuNPs), silver (AgNPs), platinum (Pt_NPs), and zinc (ZnONPs) have been described for the treatment of mastitis (100). For instance, CuNPs have shown notable inhibitory activity against various bacteria, including *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Propionibacterium acnes*, and *Salmonella typhi*. Additionally, CuNPs exhibit antifungal activity against Candida species, which

TABLE 2 Selenium role in alleviation of mastitis by improving immunity, anti-inflammatory and antioxidant status of dairy cattle.

S.No	Treatment	Biological impact	References
1	Selenium treatment (1.5 mg/kg) of *S. aureus* infected mammary gland cells (inoculation of 100 µl *S. aureus* with 7×10^8 CFU/µl)	• Mastitis induced by *S. aureus* within the mammary gland tissue of mice was driven by the upregulation of specific elements, including interleukin (IL-1β), IL-6, TNF-α, NF-κB, and MAPK pathways. • Conversely, the introduction of selenium supplementation in mice acted as a preventive measure against *S. aureus*-induced mastitis. • This was achieved by effectively inhibiting the expression levels of IL-1β, IL-6, TNF-α, NF-κB, and MAPK pathways. • Furthermore, the application of selenium treatment in mice yielded additional benefits; it not only curtailed the inflammatory response but also alleviated oxidative stress resulting from injuries to the mammary gland tissues caused by *S. aureus*.	Liu et al. (113)
2	Organic selenium (20 µg/kg body weight/day) treatment of *S. aureus* infected mammary gland cells ((2×10^7 CFU/mL)	• In the context of *S. aureus*, the initiation of inflammatory alterations occurred through the augmentation of inflammatory cytokines' expression (IL-1β, IL-6, and TNF-α), coupled with the reduction in the phosphorylation levels of MerTK, PI3K, AKT, and mTOR. • Contrastingly, selenium treatment orchestrated a different outcome: it effectively lowered the levels of IL-1β, IL-6, and TNF-α expression. Simultaneously, it bolstered the phosphorylation levels of MerTK, PI3K, AKT, and mTOR. • This intricate signaling cascade facilitated an anti-inflammatory response, enhanced antioxidant status, and ultimately mitigated the onset of mastitis in the rat population.	Chen et al. (114)
3	Selenium (0.2 mg of Se/kg) treatment of *S. aureus* ((10^5 CFU/mL) infected mammary gland cells	• In the context of *S. aureus* exposure, there was an observable increase in the expression of NLRP3, ASC, caspase-1, caspase-1 p20, and pro-IL-1β, thus intensifying the inflammatory response. • Conversely, the supplementation of selenium exerted a substantial inhibitory effect on the levels of NLRP3, ASC, caspase-1, caspase-1 p20, and pro-IL-1β. • This outcome stands as compelling evidence that selenium treatment serves as a preventive measure against *S. aureus*-induced mastitis in mice, achieved by effectively curtailing the NLRP3 level.	Bi et al. (127)
4	Selenium treatment (1.5 mg/kg) of *S. aureus* infected mammary gland cells (inoculation of 100 µl *S. aureus* with 7×10^8 CFU/µl)	• The application of selenium treatment effectively curtailed the levels of NLRP3, IL-1β, TNF-α, ASC, and caspase-1 that resulted from *S. aureus* influence within the mammary gland of mice. Moreover, Se supplementation notably bolstered the antioxidant capacity, enhanced the anti-inflammatory response, and fortified the immune status of the mice population. • Beyond this, the administration of selenium emerged as a preventive strategy against mastitis, primarily attributed to its capability to inhibit NLRP3 inflammasome activation and suppress NF-κB/MAPK pathway signaling.	Ma et al. (112)
5	Selenium (2.0 µmol Se/L) treatment of *S. aureus* infected mammary gland cells	• Dietary supplementation with selenium led to a decrease in the expression of TLR2 and the activation of the NF-κB/MAPK pathway induced by *S. aureus* in murine subjects.	Wang L et al. (138)
6	Selenium (1.5/ kg) treatment of *S. aureus* infected mammary gland cells	• *In vivo* and *in vitro* investigations demonstrated that *S. aureus* triggered inflammation, both within live organisms and primary mouse epithelial cells (MMECs) cultured in the laboratory. • This resulted in an increase in the expression levels of mmu-miR-155, IL-1β, TNF-α, and TLR2. Additionally, the phosphorylation levels within the NF-κB/MAPK signaling pathway were heightened in mammary epithelial cells of mice upon infection with *S. aureus*. • Remarkably, selenium displayed a notable capacity to suppress the elevated expression levels of mmu-miR-155, IL-1β, TNF-α, TLR2, and the NF-κB/MAPK	Zhang et al. (92)

(Continued)

(Continued)

TABLE 2 Continued

S.No	Treatment	Biological impact	References
		signaling pathways within the mammary epithelial cells of mice. These insightful findings suggest that selenium holds potential in averting mastitis in mice by mitigating oxidative stress and curtailing the inflammatory response.	
7	Selenium (1.5 mg/kg) treatment of *S. aureus* infected mammary gland cells	• Selenium intervention mitigated both oxidative stress and the inflammatory reaction. It downregulated the expression of IL-1β, TNF-α, ASC, caspase-1, and pro-IL-1β. • Additionally, selenium hindered the activation of NLRP3 within bMECs following infection with *S. aureus*.	Yang et al. (151)
8	Selenium (1.5 mg Se/kg) treatment of *S. aureus* infected mammary gland cells	• The addition of selenium curbed the inflammation provoked by *S. aureus* within the murine mammary gland. • Moreover, selenium supplementation notably decreased the quantities of myeloperoxidase (MPO), TLR2, IL-1β, TNF-α, and IL-6 within the mammary gland of mice exposed to *S. aureus*.	Gao et al. (152)
9	Selenium (1.5 mg Se/kg) treatment of *S. aureus* infected mammary gland cells	• Selenium supplementation was effective in diminishing the quantities of NF-κB and nitric oxide, while also promoting the activation of PPAR-γ activity. • These actions collectively worked to safeguard mice from developing mastitis caused by *S. aureus* infection.	Gao et al. (136)
10	Selenium administration	• Supplementing dairy cattle with selenium substantially enhanced selenium concentrations in their serum throughout the transition phase. • Additionally, there exists an inverse correlation between Se levels and milk SCC as well as IL-6 levels. • On the other hand, Se levels are positively linked to the activity of GSH-Px in periparturient dairy cattle.	Wang D et al. (122)
11	Selenium supplementation	• Low level of selenium availability in the body was associated with higher level of SCC • Selenium administration led to a reduction in milk SCC, alleviated oxidative stress, and lowered the risk of mastitis	Ceballos-Marquez et al. (153)
12	Selenite treatment	• The introduction of selenite resulted in heightened phagocyte recruitment to the infected milk compartment of the udder, augmenting both the activity of GSH-Px and the antibacterial properties of milk lactoserum. • This supplementation also curbed the *in vitro* proliferation of mastitis-causing pathogens, indicating the potential of selenium as a potent therapeutic agent in managing mastitis.	Ali-Vehmas et al. (147)
13	Selenium feeding	• Selenium enrichment improved the effectiveness of antioxidants by fostering the activity of GSH-Px in dairy cattle from Estonia. • Additionally, observations indicated that cows receiving selenium supplementation exhibited a reduced presence of pathogenic bacteria within their milk.	Malbe et al. (148); Malbe et al. (149)

(Continued)

TABLE 2 Continued

S.No	Treatment	Biological impact	References
		occurrence in periparturient dairy cattle.	

can cause mastitis. Moreover, AgNPs and AuNPs have demonstrated significant susceptibility against *S. aureus* strains isolated from clinical and subclinical mastitis cases (159). Furthermore, ZnO-NPs also exhibit antimicrobial properties against *S. aureus* and other pathogenic bacteria, such as E. coli and K. pneumoniae (160). AgNPs have also been considered for use in diseases caused by algae, which are associated with udder inflammation (161). According to research by Wernicki et al. (99), AgNPs and CuNPs may have a synergistic effect on various pathogens, making them potentially effective solutions in mastitis management.

5 The role of vitamins supplementation in mastitis alleviation

The significance of vitamins in their capacities as antioxidants and immune regulators has been extensively deliberated in the literature (16, 150, 162). Consequently, the inclusion of vitamin supplementation in mastitis control strategies has been well-documented (163–166).

5.1 Role of folic acid in mastitis prevention

The existing body of published evidence strongly supports the notion that folic acid supplementation yields notable improvements in metabolic function (167), while also playing a

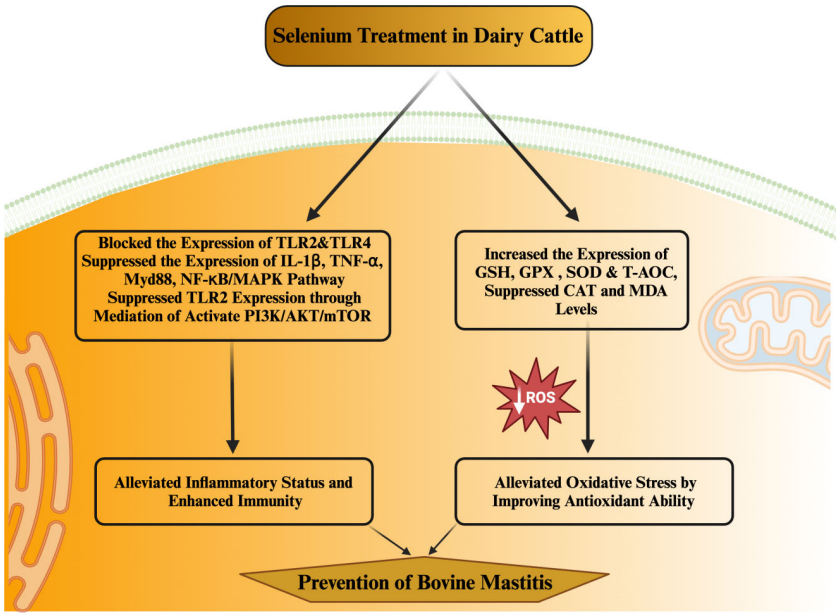

FIGURE 3
Mechanism through which selenium prevent mastitis in dairy cattle during periparturient period in dairy cattle.

crucial role in preventing oxidative stress and enhancing immune responses (168) in periparturient dairy cattle (169). The period around calving presents a vulnerability to lowered immunity in dairy cattle due to folic acid deficiency (165). Folic acid's pivotal contributions to immunity and anti-inflammatory processes have led to its exploration for mitigating bovine mastitis in periparturient dairy cattle (166, 170, 171). Recently a published research demonstrated that folic acid treatment significantly regulated LnRNA MSTRG.11108.1 in mastitic cows. The LncRNA MSTRG.11108.1 further regulated the genes (CXCL3, ICAM1, CXCL1, LHFPL2, LTF, ITGA9, and KIR3DL2) that were associated with immunity and inflammation (172). In addition, they also documented several immunity and immunity linked biological signaling pathways (B cell receptor signaling pathways, TNF signaling pathway, IL-17 signaling pathway and NF-κB signaling pathway) which is in consistent with findings reported previously (165, 173). By inhibiting the activation of MAPK and NF-κB, folic acid supplementation maintains an anti-inflammatory environment, consequently averting mastitis (170). Correspondingly, a study has recorded that administering folic acid (at a dosage of 120 mg per 500 kg of body weight) for 21 days led to the downregulation of several genes linked to immune function and inflammation (PIM1, SOCS3, ATP12A, KIT, LPL NFKBIA, DUSP4, ZC3H12, ESPNL, TNFAIP3) (165). These genes, found to be upregulated in *S. aureus*-induced mastitis during the periparturient phase in dairy cattle (174, 175) were notably downregulated.

Additionally, our prior investigation established that folic acid supplementation significantly modulated the signaling of glutathione metabolism along with its associated genes (LAP3, GSR, G6PD, GSTA4, GCLC, GPX3, PGD, IDH1, GGT1, GPX7, MGST1, and MGST2) in periparturient dairy cattle (165). Moreover, we documented that folic acid effectively enhanced dairy cattle's antioxidant capabilities and augmented their resistance to mammary gland infections during the periparturient phase. Aligning with this, (166) recently conducted an experimental study showing that *S. aureus*-induced mastitis in MAC-T cells resulted in the downregulation of the noncoding RNA associated with progenitor renewal (PRANCR). Notably, in MAC-T cells treated with folic acid, this expression exhibited an increase, suggesting folic acid's potential as a prime therapeutic agent in mastitis prevention (166). Treatment with 5 μg/mL of folic acid significantly curtailed apoptosis in Mac-T cells and offered robust defense against MRSA treatment through enhanced cytosolic DNA sensing and tightened junction signaling (173, 176). They observed the upregulation of ZBP1, IRF3, IRF7, and IFNAR2 within the cytosolic DNA-sensing pathway in folic acid-treated MAC-T cells. ZTP1, a factor associated with milk SCC (166), also assumes a critical role in activating anti-pathogenic mechanisms and inflammation (177). Furthermore, it was found that ZTP1 gene cytosolic DNA sensing pathway upregulated by folic acid treatment which has key role in activation of antipathogenic mechanism thereby enhancing the anti-inflammatory response (173). In addition, they documented that ZTP1 was significantly associated with inhibition of inflammation (177), low milk SCC and mastitis resistance (166). In light of the compiled data, we can deduce that folic acid supplementation, administered at

appropriate dosages and durations, holds promise as a valuable therapeutic resource within mastitis control strategies for periparturient dairy cattle.

5.2 Role of vitamin E supplementation in periparturient bovine mastitis prevention

Vitamin E is a fat-soluble vitamin that could protect cell membrane from the action of lipid peroxidation chain reaction (178, 179). Consequently, a study found that immune cells were prone to the effects of lipid peroxidation by ROS due to the polyunsaturated fatty acids present in their cell membranes (179, 180). Furthermore, the vitamin E combats peroxyl radicals and stops the oxidation of polyunsaturated fatty acids (PUFA). In the presence of vitamin E, peroxyl radicals react with α-tocopherol rather than lipid hydroperoxide, stopping the chain reaction of peroxyl radical formation and inhibiting further oxidation of PUFAs in the membrane (181). The oxidative stress caused by aluminum was relieved by post-vitamin E injection in rats (178, 182). Furthermore, it has been demonstrated that vitamin E is important for regulating immunity and reducing oxidative stress which are the key factors for mastitis resistance/susceptibility (182–185). Additionally, vitamin E has been shown to guard against pro-oxidant-caused harm to the integrity of the bovine mammary endothelial cell barrier (186). Mokhber-Dezfouli et al. demonstrated that intramuscular vitamin E injection could lower MDA expression and lipid peroxidation and enhance plasma's antioxidant capacity. Vitamin E supplements have been given to dairy cattle during the periparturient stage to reduce oxidative stress and maintain immunity (40). In addition, vitamin E has been extensively targeted in mastitis mitigation research in periparturient dairy cows due to its substantial role as an antioxidant and immune regulator.

Vitamin E administration has been shown to improve immunity, minimize mammary infections in dairy cattle and boost the anti-inflammatory and antioxidant functions in dairy cattle during the perinatal period (189). According to Politis et al., mastitis and oxidative stress were substantially linked to vitamin E deficiency throughout the periparturient phase (190, 191). Consequently, it has been proved experimentally that supplementation of vitamin E improved the immunity, antioxidant capacity, and anti-inflammatory ability of dairy cattle, and reduced the incidences of mastitis during the transition phase in dairy cattle (192).

Parenteral injection of 2100 mg vitamin E for 14 days before and on the day of calving significantly reduced the occurrence of periparturient mastitis in dairy cattle (87). Consistently, another study reported that supplementation of 1g vitamin E/cow/day for one month pre-calving and two months post-calving reduced the incidences of mastitis in dairy cows (193). Vitamin E has also been found effective against *E. coli* and *S. uberis* and prevented mammary gland infection during the periparturient phase in dairy cows (194, 195). Altogether, it was concluded that vitamin E is the key nutrient involved in immune regulation and relieving oxidative stress, which are the factors responsible for mastitis in periparturient dairy cattle.

5.3 Role of vitamin D supplementation in periparturient bovine mastitis prevention

Extensive research findings have demonstrated the profound impact of vitamin D (calcidiol a source of vitamin D) administration, typically within a daily dosage range of 1mg to 3mg on the regulation of various genes associated with crucial aspects of bovine immunity, inflammation, antimicrobial activity, calcium metabolism, and oxidative response (196). Notably, genes associated with immunity (CD44, ICAM1, ITGAL, ITGB1, LGALS8, SELL, NOD2, TLR2, TLR6, FOS, JUN, NFKB2), inflammation (IL1B, IL1R1, IL1RN),antimicrobial activity (CTSB, LYZ, DEFB3), calcium metabolism (TP2B1, STIM1,TRPV5, CALM3) and oxidative burst (RAC2)in periparturient dairy cattle were regulated in response to vitamin D supplementation (196). This regulatory effect is not limited to immune cells but extends to various tissues, as evidenced by the activation of 1α-hydroxylase and the subsequent regulation of calcitriol synthesis (197). Furthermore, exposure to bacterial components such as LPS and peptidoglycan has been observed to stimulate local expression of 1α-hydroxylase in peripheral blood monocytes of dairy cows. Additionally, the influence of vitamin D signaling has been elucidated in mammary gland macrophages and neutrophils when these cells are exposed to endotoxin challenge (198). *In vitro* experiments have also substantiated the role of vitamin D in up-regulating the mRNA activity of β-defensins and antimicrobial peptides in monocytes and milk somatic cell counts (199, 200). Importantly, studies have shown that vitamin D supplementation plays a pivotal role in reducing the incidence of mastitis and metritis in periparturient dairy cattle by mitigating oxidative stress and enhancing the immune response (201). Furthermore, vitamin D treatment has been associated with increased cell viability and the inhibition of *S. aureus* adhesion and invasion in bovine mammary epithelial cells, underscoring its potential significance in mastitis prevention (202). Consistently, a recent published article has demonstrated that vitamin D and its metabolites hydroxyvitamin D [25(OH)D] with a concentration of 20-400 ng/ml has shown a positive role on bovine immune cells, antioxidant response and could be consider as therapeutic agent for mastitis prevention (203–205).In light of the available literature, it is evident that vitamin D holds a central position in bolstering dairy cattle immunity and potentially alleviating bovine mastitis. However, it is worth noting that further comprehensive investigations are warranted to gain a deeper understanding of its precise mechanisms and potential therapeutic applications in this context. For ease of review, the recent findings associated with role of vitamins supplementation in boosting immune, antioxidant and anti-inflammatory response in dairy cattle during periparturient period has been summarized in Table 3. Furthermore, Figure 4 illustrates the impact of vitamin supplementation on immune, antioxidant, and anti-inflammatory responses in dairy cattle.

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TABLE 3 Role of vitamins in boosting immunity, anti-inflammation and antioxidant response in periparturient dairy cattle.

S.No	Treatment	Outcomes	References
1	Folic acid (120 mg/500 kg)/oral	Regulated immunity and suppressed inflammation via associated genes (ICAM1, GRO1 and CXCL3) and lncRNA MSTRG.11108.1 in periparturient dairy cattle	Liu X et al. (172)
2	Folic acid (10 μM FA/mL/cell culture)	Reduced cell apoptosis via elevation the expression of B-cell lymphoma-2 (BCL2) and the BCL2 to BCL2 associated X 4 (BAX4) in BMECs	Zhang J et al. (206)
5	Multivitamins and multiminerals (Zinc 40 mg/ml, Manganese 10 mg/ml, Copper 15 mg/ml, Selenium 5 mg/ml) and five ml of MV (Vitamin E 5 mg/ml, Vitamin A 1000 IU/ml, B-Complex 5 mg/ml, and Vitamin D_3 500 IU/ml)/Injection	Enhanced oxidative stress (SOD and CAT elevated), decreased anti-inflammatory cytokines (IL-4 and IL-10), increased proinflammatory cytokines (IL-1α, IL-1β, IL-6, IL-8, IL-17A, IFN-γ and TNF-α) Lower percentage of total neutrophils and immature neutrophils, higher percentage of lymphocytes as well as increased phagocytic activity of neutrophils and proliferative capacity of lymphocytes	Somagond et al. (44)
3	Folic acid (30.8 ng/mL/cell culture)	Reduced apoptosis by enhancing bcl-2/bax mRNA expression	Bae et al. (176)
4	Vitamin E and folic acid	Regulate immunity, antioxidative stress and relieved inflammatory responses and prevent mastitis	Khan et al. (16) and Xiao et al. (36)
6	Folic acid (5 μg/mL/cell culture)	Mediated the expression alteration in lncRNAs linked to toxin metabolism and inflammation to fight against *S. aureus* infection	Mi et al. (166)
7	Folic acid (120 mg/500 kg)/oral	Relieved oxidative stress, enhanced immunity and anti-inflammatory status, reduced SCC level and improved milk production	165
8	Folic acid alone or in combination with vitamin B12/(120 mg/500 kg)/oral	Enhanced the perimarturient dairy cattle health and production performance (enhanced immunity and milk production)	167, 169
9	Calcidiol 3 mg/d (3mg) prepartum	Regulated bovine immunity, anti-inflammatory response, antimicrobial activity leukocytes, pathogen recognition efficacy, calcium metabolism, and antioxidative status of periparturient dairy cattle	Vieira-Neto et al. (196)
10	25-hydroxyvitamin D3 (3 mg/oral)	Lowered the level of SCC and enhanced T-AOC, total SOD, CAT, immunoglobulin A and immunoglobulin G and decreased MDA and TNF-α. Reduced the susceptibility of periparturient dairy to mastitis	Xu et al. (207)
11	Dietary protein levels (10.3% or 12.2%), vitamin A levels (0 or 110 IU/kg body weight),	Lowered the level of SCC and improved ant-inflammatory response in periparturient dairy cattle	Agustinho et al. (69)
12	The supplementation of vitamin A, 10^5 IU + zinc sulphate, 60 ppm+ vitamin E, 2500 IU) in compounded concentrate DM (100 g)/oral	Lowered milk SCC and enhanced total immunoglobulins in colostrum Lowered oxidative stress and cortisol levels and higher immune response Improved the phagocytic activity of neutrophils of periparturient dairy cattle	Alhussien et al. (70)

5 The effect of rumen-protected amino acids on immune function, oxidative and anti-inflammatory status of dairy cattle

The cellular detoxification process is facilitated by GSH through the action of GST and the neutralization of hydrogen peroxide by

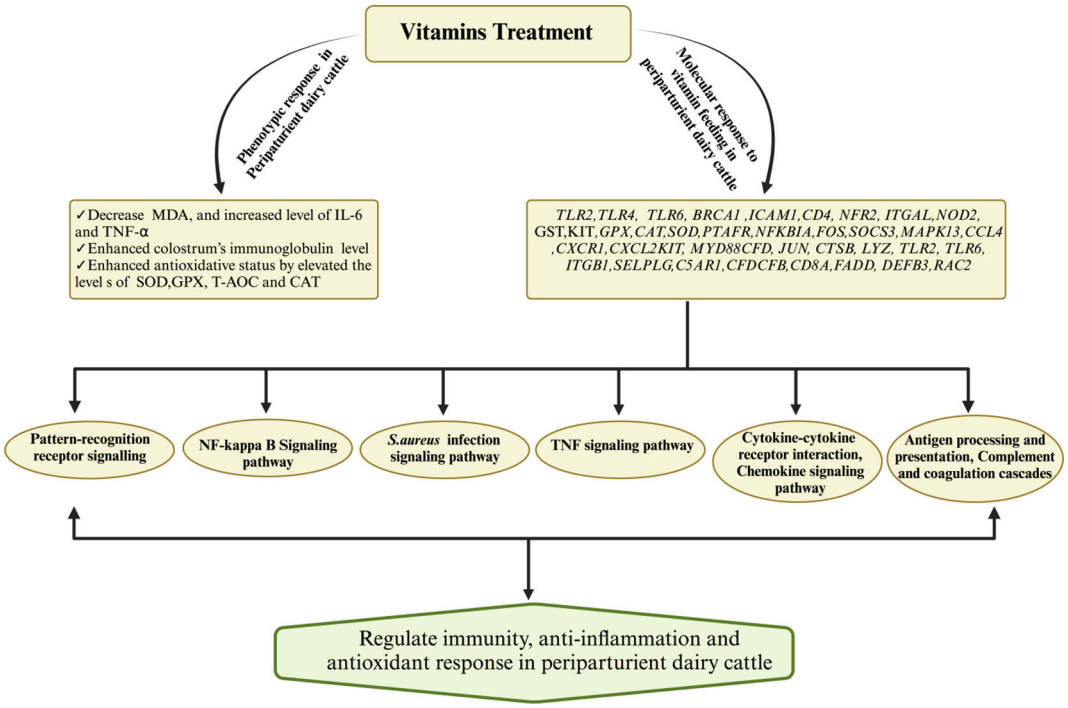

FIGURE 4
Mechanisms governing the regulation of immunity, antioxidant, and anti-inflammatory responses for the prevention of mastitis in periparturient dairy cattle through vitamin supplementation. Vitamin supplementation demonstrated an augmented antioxidative status by upregulating key antioxidant genes (SOD, CAT) while concurrently downregulating the levels of MDA. Additionally, vitamins exhibited regulatory effects on immunity-related and inflammatory-associated genes and their associated signaling pathways, thereby substantiating their pivotal role in mastitis resistance or susceptibility.

GSH-Px. The GSTs (EC 2.5.1.18) are pivotal antioxidant enzymes responsible for the regulation of cellular redox equilibrium, as documented by several studies (208–210). Recent evidence underscores the involvement of methionine in glutathione synthesis (211), thereby potentially enhancing the antioxidant capacity in animals and their products. This notion is corroborated by research demonstrating that methionine, in conjunction with choline, elevates glutathione and amino acid levels in perinatal dairy cows (212). Furthermore, methionine supplementation has been found to augment very-low-density lipoprotein (VLDL), facilitating the circulation of vitamin E (213). Consequently, the detrimental impact of lipid peroxidation by-products, such as MDA, can be mitigated through the administration of rumen-protected amino acids (213). Additionally, the control of ROS by antioxidant systems, categorized into enzymatic and non-enzymatic components like metabolites, has been extensively discussed in previous studies (210, 214).

Dysregulated immune function, notably in early lactation dairy cows, has been observed, adversely affecting neutrophils, circulating monocytes, and lymphocytes (6, 215 55, 216). It is postulated that complete metabolic adaptations are required to cope with the substantial nutrient demands associated with lactation initiation, contributing to immunological dysfunction (19, 217, 218). A noteworthy finding is that prolactin blockade leads to an increase in oxidative burst activity in neutrophils, resulting in an initial reduction in milk production and nutritional requirements, along with a subsequent decline in lymphocyte proliferation (219).

Empirical investigations have consistently demonstrated that methionine supplementation exerts favorable effects on the anti-inflammatory and antioxidant status in periparturient dairy cattle (220–222) and neonatal calves (223). A recent study conducted by Hu et al. has reported that the supply of methionine and arginine significantly modulates milk protein synthesis, thereby alleviating potential inflammatory and pro-oxidant conditions in transition dairy cattle (224). Moreover, emerging research has highlighted the significance of methionine supplementation in conjunction with arginine in conferring anti-inflammatory effects and enhancing the antioxidant status in transition dairy cattle. Notably, Dai et al. (225) and Batistel et al. (222) have reported the beneficial impact of methionine and arginine co-supplementation. Additionally, Abdelmegeid et al. (226) documented that the combination of choline and methionine effectively regulates antioxidative mechanisms, resulting in heightened anti-inflammatory and cytoprotective responses against oxidative stress in neonatal Holstein calves. Consistently, Zhou et al. (227) demonstrated that choline and methionine supplementation improved immunometabolic status, bolstered blood polymorphonuclear leukocyte phagocytosis capacity, promoted anti-inflammatory responses upon pathogenic challenges, and elevated antioxidative capacities in peripartal cows. In a relevant experiment, a group of cows was subjected to either methionine supplementation alone or

in combination with lysine. Wang H et al. (223) found that the offspring (calves) of cows receiving rumen-protected amino acids exhibited heightened passive immunity, characterized by increased immunoglobulin G concentrations and superior growth rates compared to their counterparts in the unsupplemented amino acid group. Furthermore, they documented that methionine supplementation upregulated the expression of antioxidant-related genes, such as SOD and GSH-Px and conferred cytoprotective effects against hyperthermia (228, 229). The synergistic effects of methionine and arginine supplementation have also been observed in the regulation of immunity and the mitigation of oxidative stress induced by bacterial LPS in BMECs (225). Dai et al. (225) reported that bacterial LPS significantly down-regulated the expression of key genes associated with antioxidant responses, such as NFE2L2, NQO1, GPX1, ATG7, and GPX3, while increasing the levels of SOD2 and NOS2 in BMECs. Folic acid was found to enhance antioxidant activity, reduce the expression of inflammation-related genes, and improve udder health in dairy cattle (225). Additionally, when BMECs were challenged with gamma-d-glutamyl-meso-diaminopimelic acid (iE-DAP), a component of bacterial cell walls, it induced inflammatory changes and oxidative stress, which were effectively mitigated by arginine and methionine treatment (230). Similarly, another study demonstrated that glutamine treatment provided protective effects against the adverse effects of iE-DAP in BMECs (231). Furthermore, iE-DAP was found to elevate the expression of inflammatory markers, including NOD1, inhibitor of nuclear factor-κB (NFKBIA, IκB), nuclear factor-κB subunit p65 (RELA, NF-κB p65), IL-6, and interleukin-8 (IL-8) in cell culture (231). However, when cells treated with iE-DAP were subsequently exposed to glutamine, this intervention led to the suppression of the NOD1/NF-κB pathway and the enhancement of antioxidant protein levels (231). This aligns with previous findings that amino acids play a pivotal role in NO regulation in mammary cells, thereby exerting antibacterial activity against LPS during inflammation (232–234). *In vitro* experiments have consistently demonstrated that methionine supplementation effectively attenuates apoptosis, necrosis, and lipid peroxidation in bovine mammary gland cells (228, 229, 235). Moreover, these studies have shown that arginine and methionine co-supplementation enhances the expression of antioxidative genes and elevates the NFE2L2 signaling pathway in mammary cells, a crucial component of the cellular antioxidant defense system (236). Lan et al. (237) conducted experimental research revealing that pretreatment with 2 mM Met-Met had the capacity to mitigate the elevated levels of specific inflammatory markers following exposure to 1 μg/mL LPS. This included a reduction in TNF-α, IL-1β, and IL-8. Furthermore, their investigation indicated that the genes commonly affected by this treatment were primarily associated with the NF-κB, MAPK, and IL-17 pathways. Notably, the suppression of NF-κB, P38, and JNK by Met-Met appeared to occur through the Janus kinase 2-signal transducers and activators of transcription 5 (JAK2-STAT5) pathway (237). Additionally, it was observed that the Met-Met-induced reduction in LPS-triggered activation of p-IκB, NF-κB, and JNK was reversed in the presence of a JAK2 inhibitor, highlighting the intricate interplay between Met-Met and these signaling pathways (237). Furthermore, a concise summary of research endeavors investigating the impact of rumen-protected amino acids on immune function, oxidative stress, and anti-inflammatory responses in dairy cattle has been thoughtfully compiled in Table 4 for your reference and elucidation.

TABLE 4 Summary of studies investigating the effect of rumen-protected amino acids supplementation on immune function, oxidative and anti-inflammatory status in dairy cattle.

S.No	Amino acid Administration	Main outcomes	References
1	Methionine and lysine supplementation (107g/ twice a day/7 weeks/oral)	• Attenuated the severity of SCC and BCS, thereby mitigating the risk of clinical mastitis occurrence.	Abreu et al. (238)
2	Methionine/at a rate of 0.09% and 0.10% of DMI/ oral/28 day before parturition till 60 days after parturition (88 days)	• Notable enhancement in plasma biomarkers, following improved liver function, reduced oxidative stress and inflammation, as well as enhanced oxidative burst and neutrophil phagocytosis.	Batistel et al. (222)
3	Increase methionine (175 μg/mL) and lysine (175 μg/mL) ratio 1:2.5 respectively	• Modulated the expression of genes associated with immunity, anti-inflammation, and antioxidation in LPS-challenged BMECs. • Elevated the expression of genes such as NFE2L2, NQO1, GPX1, GPX3, SLC36A1, SLC7A1, SOD2, NOS2, and concurrent decreased expression of RELA, IL1B, NF-kb, and CXCL2.	Dai et al. (225)
4	5-10 gm zinc methionine/ head/day/oral	• Reduced the SCC levels and decreased the required mastitis treatment duration with antibiotics	Gaafar et al. (239)
5	Methionine at a rate of 0.09% and 0.10% of DMI/ oral/28 day before	• Upregulated the expression of genes involved in the metabolism of	Han et al. (240)

(Continued)

TABLE 4 Continued

S.No	Amino acid Administration	Main outcomes	References
	parturition till 60 days after parturition (88 days)	antioxidants, notably increasing the expression level of NFE2L2, a prominent transcription factor associated with antioxidant response.	
6	Methionine at a rate of/ 0.08% of DM/d/oral	• Reduced inflammatory changes, improved antioxidant status followed by augmentation of immune responses, thereby reducing the susceptibility to infections in transition neonatal calves	Jacometo et al. (241); Jacometo et al. (242) & Jacometo et al. (243)
7	2 mM methionyl-methionine treatment	• Mitigated the inflammatory alterations induced by LPS through downregulation of the expression of inflammatory-associated genes, including *TNF-α, AP-1, MCP-1, Jak2, IL-1β*, and *IL-8*, along with the inhibition of key signaling pathways such as *JAK2-STAT5, NF-κB, MAPK*, and *IL-17* in BMECs	Lan et al. (237)
8	2 mM methionyl-methionine treatment	• Significantly reduced the expression of inflammatory linked genes such as *IL-8*, *TNF-α*, *AP-1*, and *MCP-1* induced by LPS in BMECs	Lan et al. (244)
9	Rumen-protected lysine (10 g of digestible lysine/ cow per day) and methionine (4 g of digestible Methionine/cow)	• Reduced the levels of BHB, improved BCS, and lowered SCC, which collectively contributing to enhanced udder health in periparturient dairy cattle	Lee et al. (245)
10	Hydroxyselenomethionine supplementation (0.5 mg/oral)	• Significantly elevated the levels of antioxidative	Li et al. (246)

(Continued)

TABLE 4 Continued

S.No	Amino acid Administration	Main outcomes	References
		status and reduced the risk of periparturient mastitis in cattle	
11	Methionine supplementation at a rate of 0.09%/oral	• Enhanced the mRNA expression of genes associated with antioxidative status and the metabolism of GSH, thereby improving cellular antioxidant defenses.	Liang et al. (247)
12	60 g/d of NALM acetyl-l-methionine (NALM)/cow	• Ameliorated oxidative stress in mid-lactating dairy cows, as evidenced by increased concentrations of total plasma protein and globulin, concomitant with a reduction in plasma MDA concentration.	Liang et al. (248)
13	Methionine and choline treatment/cell culture	• Significantly enhanced the expression of genes linked to immunity and anti-inflammatory responses, while concurrently reduced oxidative stress in bovine PMNLs	Lopreiato et al. (249)
14	Methionine supplementation at the rate of 0.19 or 0.07% DMI	• Enhanced whole blood neutrophil phagocytosis followed by improved immune defense system • Reduced oxidative stress and improved antioxidant response in dairy catle • The improved immunity and antioxidant status were associated with reduced risk of mastitis	Osorio et al. (250)
15	Methionine supplementation at the rate of 0.19 or 0.07% DMI	• Triggered a series of positive metabolic changes with a notable	Osorio et al. (211); Osorio et al. (220)

(Continued)

TABLE 4 Continued

S.No	Amino acid Administration	Main outcomes	References
		enhancement of 1-carbon metabolism, showcasing its impact on fundamental metabolic processes. • Antioxidant status was improved, as evidenced by elevated levels of liver GSH. • Decreased concentrations of plasma biomarkers associated with inflammation, indicating its potential role in mitigating inflammatory responses and promoting overall physiological well-being	
16	Zn-Meth supplementation (1 g/d Zn/oral)	• **Reduced inflammatory changes, enhanced immunity and lowered the SCC following improved** udder health in transition dairy goats	Salama et al.
17	Increase methionine (175 µg/mL) and lysine (175 µg/mL) ratio 1:2.5 respectively	• Regulated the **AKT1 and mTORC1 Signaling** which are pivotal in cell growth, proliferation, and immune responses. • In addition elevated the expression of genes associated with anti-inflammatory responses and metabolic regulation (***PPARG***), anti-apoptotic activity (CL2L1) and cell growth and immunity (***MAPK1 MTOR, RPS6KB1, BAX, EIF4EBP1*** **and** ***JAK2***)	Salama et al. (252)
18	Methionine (30 g/d of Mepron)/ oral supplementation	• Improved immune system's cellular defense mechanisms by	Soder and Holden, (253)

(Continued)

TABLE 4 Continued

S.No	Amino acid Administration	Main outcomes	References
		enhancing population of T lymphocytes in the bloodstream. • Reduced the level of SCC, which shows methionine supplementation could significantly prevented mastitis	
19	15 g/d RPC + 15 g/d RPM from 21 days prepartum to 21 days postpartum.	• Enhanced CD4 +/CD8+ T, GSH-Px, T-AOC, decreased the plasma concentrations of NEFA, BHBA, total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) • In addition, lymphocyte ratio, immunity and antioxidative status were improved	Sun et al. (213)
20	Methionine (8.0 and 12.0 g/d) and choline (12.4 g/d) supplementation	• Effectively mitigated the hyperactive response of IL-1β during an LPS challenge. This suggests that methionine improved the body's ability to regulate inflammatory processes, potentially reducing the risk of excessive inflammation and its associated negative effects. • Relieved oxidative stress and improved postpartum neutrophil and monocyte phagocytosis capacity. • In addition phagocytosis and oxidative burst activity was improved. This means that immune cells were more effective in generating reactive oxygen species to	Vailati-Riboni et al. (254)

(Continued)

TABLE 4 Continued

S.No	Amino acid Administration	Main outcomes	References
		destroy pathogens, further strengthening the immune response.	
20	Infusion of arginine (3gm/h for 5 days) protected the dairy cattle from LPS challenge (0.033 µg/kg per h)	• Relieved oxidative stress and inflammatory changes caused by LPS in dairy cattle. Arginine infusion played a role in mitigating inflammation triggered by LPS. • Suppressed the LPS induced NOS and reduced the LPS-binding protein levels. This is significant because LPS-binding protein is associated with the body's recognition of bacterial endotoxins, and reducing its levels may contribute to a reduced risk of systemic inflammation.	Zhao et al. (255)
21	Infusion of arginine (3gm/h for 5 days) protected the dairy cattle from LPS challenge (0.033 µg/kg per h)	• Enhanced the body's antioxidant defenses. This could be due to its properties as a precursor to NO, which has antioxidant effects. • Enhanced the level of TAC which suggests that arginine contributes to the body's ability to counteract oxidative stress during inflammation. • Increased the expression of GSH and inhibited the MDA level in LPS challenged dairy cattle	Zhao et al. (256)
22	Methionine and choline supplementation (at a rate of 0.08% of DM/oral)	• Regulates taurine synthesis, which is involved in supporting antioxidant defenses and stabilizing cell membranes.	Zhou Z et al. (221)

(Continued)

TABLE 4 Continued

S.No	Amino acid Administration	Main outcomes	References
		• Being a precursor for GSH, play a key role in enhancing antioxidant status. • Reduced inflammatory and chances of udder infections in periparturient dairy cattle	
23	Methionine supplementation at 0.08% of DMI/oral l	• Improved biochemical pathways, including DNA methylation, which can influence gene expression. Increased methionine availability can potentially affect the expression of genes related to different biological processes (immunity, inflammation and oxidative stress) • Strengthening the immune system by upregulating the expression of genes associated with immunity. • Enhanced the body's antioxidant defenses by influencing the expression of antioxidant linked.	Zhou et al. (212)
24	Methionine supplementation at 0.08% of DMI/oral	• Significantly decreased the expression of genes associated with inflammation and oxidative processes and improved the udder health in periparturient dairy cattle	Zhou et al. (227)

6 Future research direction

While this review has synthesized existing knowledge regarding the role of amino acids, vitamins, and trace minerals in mitigating periparturient mastitis, several avenues for future research emerge. Further investigation into the intricate mechanisms underlying the interactions between these nutrients, oxidative stress, and immune modulation will deepen our understanding of their synergistic effects. Incorporating molecular and cellular approaches can unravel the specific pathways through which these nutrients

influence immune responses and oxidative balance. Additionally, longitudinal studies assessing the long-term effects of targeted supplementation on mastitis incidence, milk quality, and overall cow health are essential to validate the efficacy of these strategies under practical farming conditions. Furthermore, understanding the interplay between genetic factors, environmental conditions, and nutritional interventions can provide insights into personalized approaches for mastitis prevention. Moreover, exploring novel delivery mechanisms for these nutrients, such as innovative formulations or precision feeding, could optimize their absorption and utilization within the complex physiological milieu of periparturient dairy cattle. In conclusion, future research endeavors should focus on unraveling the nuances of nutrient-nutrient interactions, delineating precise molecular mechanisms, and translating these findings into practical strategies that empower dairy farmers to effectively manage periparturient mastitis, bolstering animal health and farm productivity.

7 Conclusion

In light of the intricate interplay between immune suppression, oxidative stress, and metabolic perturbations during the periparturient period, this comprehensive review underscores the paramount importance of proactive nutritional strategies in mitigating bovine mastitis. The critical vulnerabilities arising from negative energy balance, oxidative stress, and compromised immune responses underscore the need for targeted interventions to enhance udder health and overall productivity in dairy cattle. The exploration of trace minerals, vitamins, and amino acids as key mitigation factors offers promising avenues for addressing periparturient bovine mastitis. These nutritional components have demonstrated significant potential in bolstering antioxidant defenses, modulating immune responses, and preventing oxidative damage. The multifaceted roles of trace minerals, particularly copper, selenium, and calcium, have been deliberated in the context of mastitis control. Similarly, the established impact of vitamins, such as vitamin B12 and vitamin E, in enhancing metabolic function and immune responses underscores their potential in ameliorating mastitis susceptibility. Furthermore, amino acids' pivotal role in maintaining cellular oxidative balance through their participation in crucial biosynthesis pathways presents a novel perspective in combatting mastitis-related challenges. The insights gained from this review highlight the need for a holistic approach that encompasses these nutritional factors to enhance udder health and mitigate the risks associated with periparturient mastitis.

Author contributions

MK: Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. BH: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. XK: Data curation, Writing – review & editing. YC: Data curation, Writing – review & editing. HL: Data curation, Writing – review & editing. QU: Data curation, Writing – review & editing. IK: Data curation, Writing – review & editing. AK: Data curation, Writing – review & editing. WC: Data curation, Writing – review & editing. CW: Conceptualization, Data curation, Funding acquisition, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Tomato-made edible COVID-19 vaccine TOMAVAC induces neutralizing IgGs in the blood sera of mice and humans

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Plant-based edible vaccines that provide two-layered protection against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outweigh the currently used parenteral types of vaccines, which predominantly cause a systemic immune response. Here, we engineered and selected a transgenic tomato genotype (TOMAVAC) that stably synthesized an antigenic S1 protein of SARS-CoV-2. Two-course spaced force-feeding of mice with ≈5.4 μg/ml TOMAVAC increased up to 16-fold the synthesis of RBD-specific NAbs in blood serum and the significant induction of S-IgA in intestinal lavage fluid. In a surrogate virus neutralization test, TOMAVAC-induced NAbs had 15–25% viral neutralizing activity. The results suggested early evidence of the immunogenicity and protectivity of TOMAVAC against the coronavirus disease 2019 (COVID-19) infection. Furthermore, we observed a positive trend of statistically significant 1.2-fold (average of +42.28 BAU/ml) weekly increase in NAbs in the volunteers' serum relative to the initial day. No severe side effects were observed, preliminarily supporting the safety of TOMAVAC. With the completion of future large-scale studies, higher-generation TOMAVAC should be a cost-effective, ecologically friendly, and widely applicable novel-generation COVID-19 vaccine, providing two-layered protection against SARS-CoV-2.

KEYWORDS

SARS-CoV-2, Edible vaccine, RBD-specific neutralizing antibodies, viral neutralizing activity, efficiency, safety

Introduction

Vaccination is considered the most effective way to treat and prevent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is currently infecting more than 650 million people around the globe (1–5). Presently, most commercial vaccines are parenteral and administered intramuscularly, inducing only systemic immunity (6–9). However, for air droplet-spreading SARS-CoV-2, parenteral vaccines are less effective for the protection of viral transmission (10, 11), enabling its successful replication in the epithelial cells of the upper respiratory tract. Because of ineffective primary-entrance cell viral neutralization by circulating IgG (NAbs), there is a risk of developing and spreading new SARS-CoV-2 variants of concern (VOCs) (10). The production of parenteral vaccines is resource-intensive, ecologically unfriendly, and requires special conditions for transportation, storage, and use (5, 8, 12–14).

Alternatively, mucosal vaccines, including plant-based edible types, activate both mucosal and systemic immune responses, providing adequate and long-term protection against viral transmission, disease progression, and antiviral prophylaxis (10, 11). They also offer an opportunity for targeted folding and post-translational modifications of antigenic proteins with the desired properties (5, 15). Several attempts have been made toward developing plant-based COVID-19 vaccines (5, 16–20), and many plant-carrier-based examples exist for various infectious diseases (8, 9, 12–14). Here, we present the results of our efforts in developing transgenic tomato plants expressing the RBD subunit of the S1 protein of SARS-CoV-2 with early evidence for the immunogenicity, neutralizing activity, and safety of the first tomato-based edible vaccine (TOMAVAC) against COVID-19.

Methods

TOMAVAC plant development

A binary pART27 vector bearing the custom synthesized *S1* gene (Figure 1) of SARS-CoV-2, driven by the CaMV 35S viral promoter, was constructed and transformed into *A. tumefaciens* strain LB4404 (21). The *in fruit* transformation of tomatoes (*Solanum lycopersicum* cv. Bella-Rossa) was carried out by modified methods (22, 23). The success of the transformation into *A. tumefaciens* strain LB4404 and tomato genomes were confirmed and monitored by PCR amplification of the S1 gene from the genomic DNA of the transformants. Transgenic plant development and characterization, genomic DNA isolation, PCR, and qPCR analyses, including relative expression and qPCR-based transgene copy number identification, were carried out as described in our previous work (24), optimizing for tomatoes. S1-specific protein synthesis in transgenic plants was determined using western blotting and ELISA (Supplementary Tables 1–6). The use of plant material and the performance of experimental research on such plants in the study comply with guidelines for conducting genetic engineering research in the Center of Genomics and Bioinformatics, Academy of Sciences of Uzbekistan. The detailed step-by-step protocols can be found in the Supplementary material section of this article.

TOMAVAC feeding in mice

In total, 100 ($n = 100$) 20-day-old outbred ICR CD-1 mice were kept in plastic cages and fed between 7–8 a.m. and 4–5 p.m. daily on a standard diet (Supplementary Methods) adaptation. After NAbs analysis of serum and initial verification of the noninfected status of the mice (sacrificing $n = 4$), 96 healthy mice ($n = 96$) were divided into three equal groups ($n = 24$ per group, male and female). Groups were (1) the TOMAVAC group, force-fed with 1 ml of homogenate (equivalent to ≈0.77 μg S1 protein) of the transformation event 4; (2) the untransformed tomato group, force-fed with 1 ml of homogenate of the original cv. Bella Rossa®; (3) a control group fed only with a standard diet; and (4) a positive control group parenterally vaccinated with AstraZeneca (ChAdOx1 NCoV-19; Cambridge, United Kingdom). A 7-day-spaced, two-dose force-feeding with tomatoes was performed using a syringe before every morning feeding (Figure 3A). A two-dose (day 0 and day 14), 7-day-spaced intramuscular AstraZeneca (75 μl of working solution) immunization was carried out in mice in compliance with asepsis rules. Each dose contained ≈16×10^6 infectious units in a 75 μl of injection solution. To do this, 10 μl of the original AstraZeneca vaccine was diluted to 1,000 μl by adding sterile standard saline solution. Then, the obtained solution was diluted to a final concentration of 16×10^6 infectious units by sterile solution.

For the analyses of NAbs, blood samples and intestinal lavage fluid were taken on day 14 ($n = 24$, six mice per group), day 28 ($n = 24$, six mice per group), day 42 ($n = 24$, six mice per group), and day 56 ($n = 16$, four mice per group). Mouse experiments were conducted with ethics board approval (no. 3/30062022) and in accordance with the US National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (25). Animal experiments were conducted at the Drug Standardization Scientific Center, Pharmaceutical Institute, Tashkent, Uzbekistan (Supplementary Protocol).

Determination of NAbs titer and neutralizing ability

RBD-specific serum IgG was assessed in three different groups using the SARS-CoV-2 (2019-nCoV) Spike RBD Antibody Titer Assay Kit (Mouse) according to the manufacturer's instructions (Krishgen Biosystems, Cerritos, California, United States). The neutralizing activity of RBD-specific antibodies was determined in blood serum on days 42 and 56 post-vaccination using the SARS-CoV-2 surrogate Virus Neutralization Test (sVNT) kit according to the manufacturer's instructions (GenScript, Piscataway, New Jersey, United States). RBD-specific secretory immunoglobulin A (S-IgA) quantified by ELISA using the Mouse Anti-2019 nCoV(S)IgA ELISA Kit (Wuhan Fine Biotech Co., Ltd., Wuhan, China) according to the manufacturer's instructions (see Supplementary Methods).

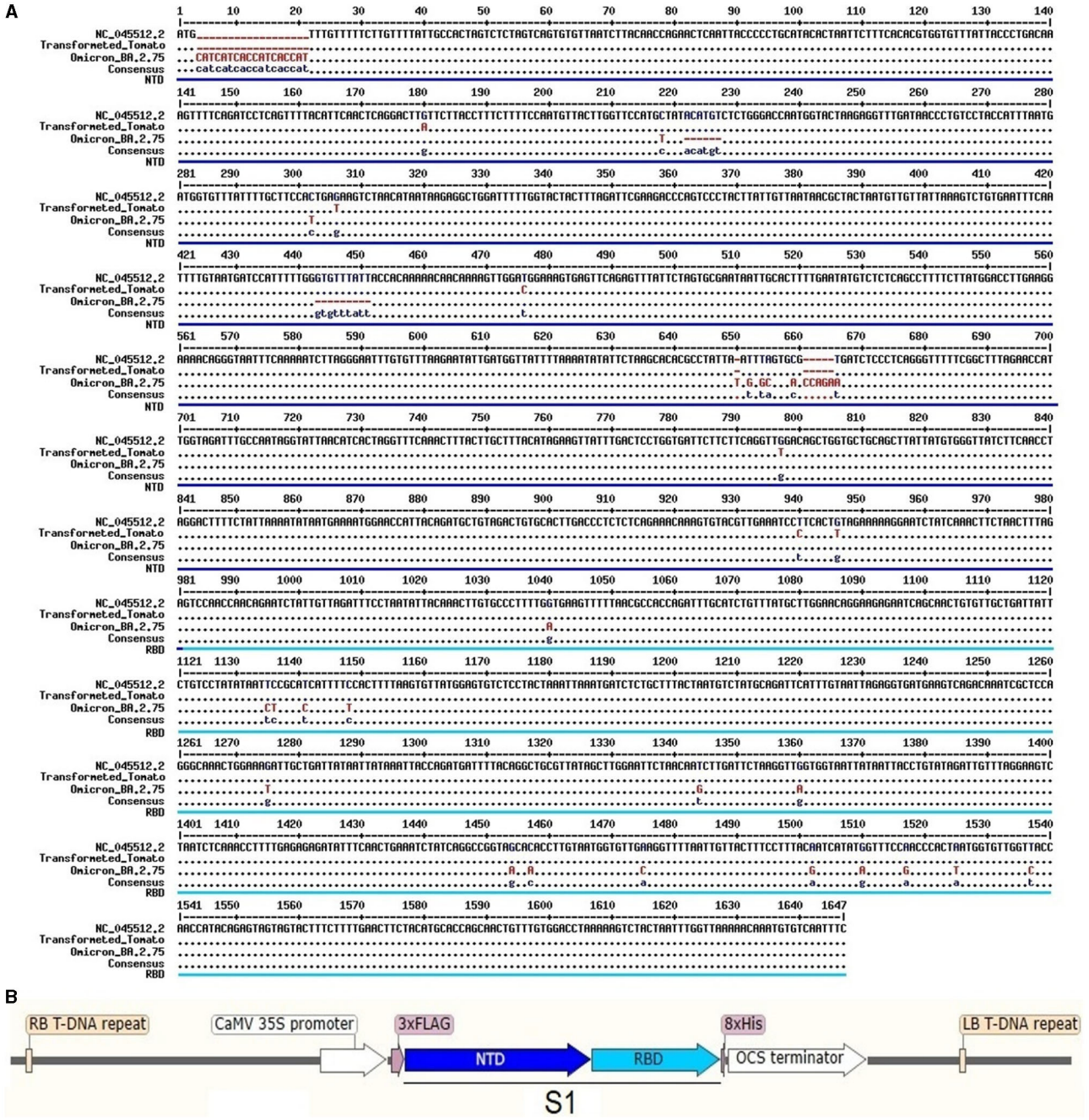

FIGURE 1
The S1 gene sequence of SARS-CoV-2 was used to construct a binary vector: **(A)** sequence alignment and **(B)** a scheme of vector construction used for tomato transformation.

Proof-of-concept human consumption study

All 14 healthy adults aged 30–52 years volunteered to participate in a human proof-of-concept experiment. All experiments were designed and carried out in accordance with the methods of relevant guidelines and regulations. The ethical board of the Center of Genomics and Bioinformatics, Academy of Sciences of Uzbekistan, approved the experimental protocols. Informed consent was obtained from all subjects and/or their legal guardian(s). Following the protocol approval, volunteers were asked to be randomly divided into two groups: (1) those who were invited to take TOMAVAC (n = 7) before dining and (2) those who were invited to have their regular food (n = 7). Both groups were instructed not to have any other vaccinations during the experiments

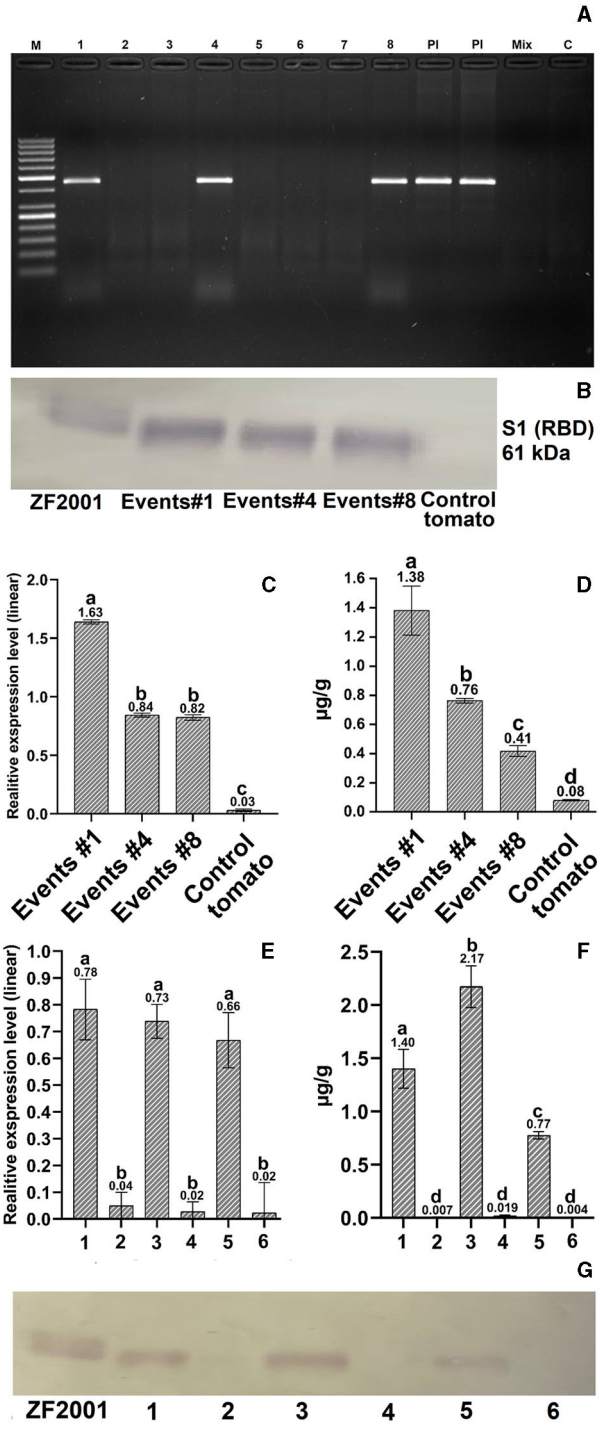

FIGURE 2
Molecular evaluation of transformed tomatoes. **(A)** PCR analysis: M – GeneRuler 50 bp DNA Ladder (Thermo Fisher, Waltham, MA, United States); 1–8 – transformed tomato plant DNA; Pl – S1 plasmid DNA (a positive control); Mix – Master mix without DNA (a negative control); C – a negative untransformed tomato plant DNA control. **(B, G)** Western blot quantification of expressed protein in transgenic tomatoes. RBD protein (ZF-UZ-VAC200123,28 with 61.7 kDa) and untransformed tomatoes were used as positive and negative controls **(C, E)** Relative expression analysis of transformed T1-generation tomato plants and tissues. The untransformed tomato was used as a negative control. **(D, F)** ELISA analysis of transformed T_1-generation tomato plants: leaf tissue (1), unripened *(Continued)*

FIGURE 2 (Continued)
green tomato (2), ripened red tomato (3), and control tissues from untransformed tomato (2, 4, and 6). Different letters designate significant differences at the $p \leq 0.05$ level of one-way ANOVA or Kruskal-Wallis one-way nonparametric ANOVA (Supplementary Tables 1, 3), with similar letters reporting nonsignificant differences. Refer to Supplementary Figures S1–S3 for the original gel images for **(A, B, G)**, cropped for presentation purposes.

(Supplementary Protocol). All procedures were explained clearly to all participants, and their approval of using the collected data for "research purposes" was obtained. Participants were informed about blood test results but deidentified for reporting purposes.

Briefly, the experimental group consumed 50 g of TOMAVAC on an empty stomach daily for 3 consecutive days, 20–30 min before dining. Blood samples were taken from all participants before consumption (day 0) and on days 7, 14, and 21. The NAbs levels were tested at the Institute of Immunology and Human Genomics, Uzbekistan, using the automatic chemiluminescence immunoassay analyzer MAGLUMI series, according to the manufacturer's instructions for human sera (Snibe Diagnostic, Pingshan, China).

Statistical analysis

Expression and ELISA data in tomatoes were analyzed using an ordinary one-way ANOVA with Tukey's multiple comparisons. For mouse data, after checking the normal distribution using the Shapiro-Wilk test or data normalization using the log10 transformation, an ordinary one-way ANOVA was used with the recommended Tukey's multiple comparisons. A nonparametric Kruskal-Wallis (KW) one-way ANOVA was also applied to determine the statistical significance of abnormal data and seek additional statistical evidence to judge ordinary ANOVA findings. In the human study, due to data abnormality, the nonparametric Friedman's ANOVA test, followed by Dunn's multiple comparisons, was performed comparing the initial NAbs mean ranks to the repeated measurement mean ranks of NAbs from days 7, 14, and 21. Data analysis and visualization were performed using GraphPad Prism version 8.0.1 for Windows (www.graphpad.com; GraphPad Software, San Diego, California, United States). A threshold of $p < 0.05$ was considered significant (Supplementary Tables 7–9).

Ethics of the research

All methods were performed in accordance with the relevant guidelines and regulations reviewed and approved by the Institutional Ethics Board of the Center (Supplementary Protocol). The use of plants in this study complied with relevant institutional, national, and international guidelines and legislation. All animal results are reported following the ARRIVE guidelines.

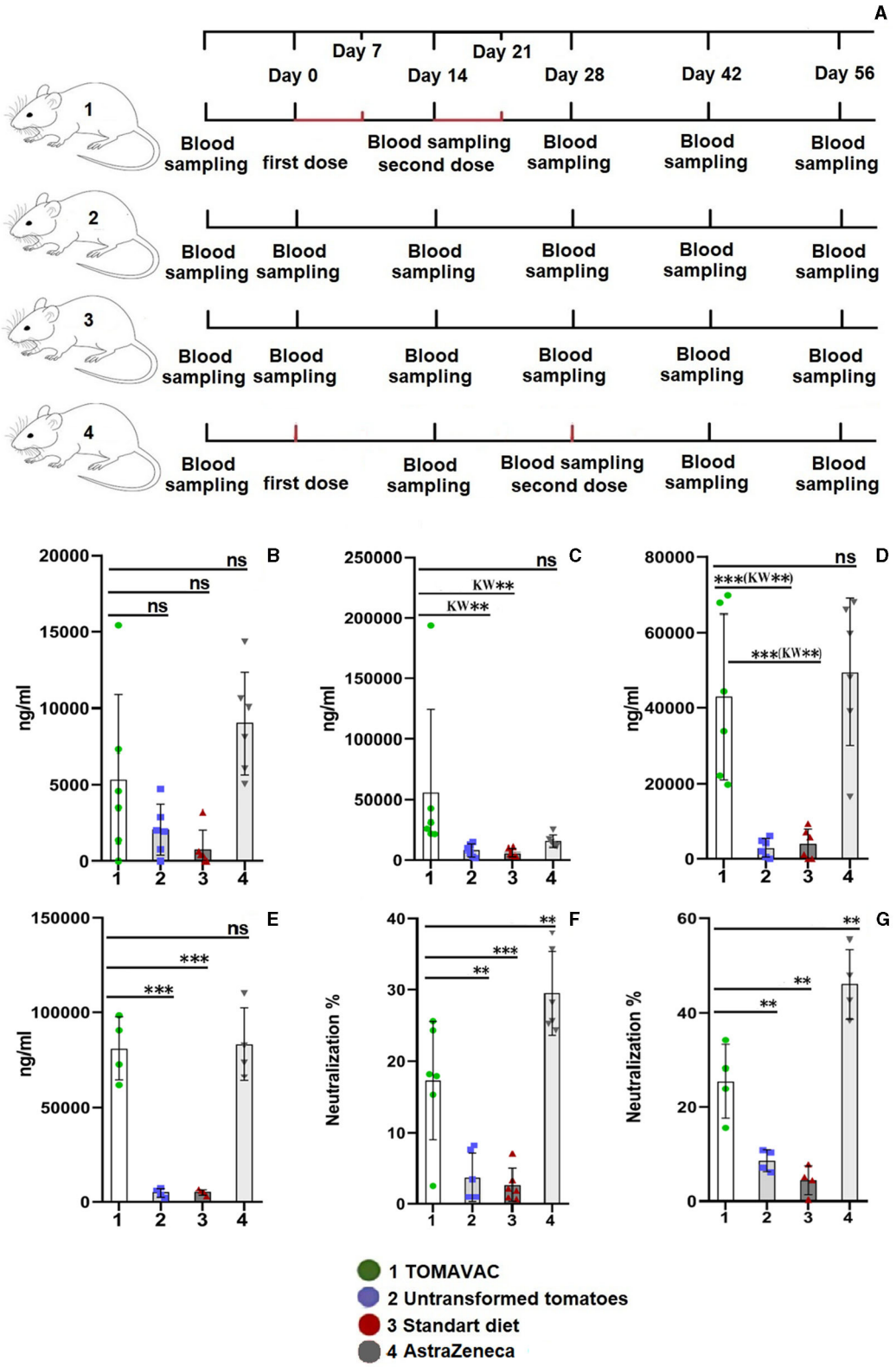

FIGURE 3
Titer and neutralizing activity of RBD-specific serum IgG in mice after oral vaccination. (A) – animal experiment scheme; **(B)** – titer on day 14 postvaccination; **(C)** – titer on day 28 postvaccination; **(D)** – titer on day 42 postvaccination; **(E)** – titer on day 56 postvaccination; **(F)** – the neutralizing activity of NAbs on day 42 postvaccination; and **(G)** – neutralizing activity on day 56 postvaccination. Statistical significance level (**–≤0.01, and ***–≤0.001) from ordinary one-way ANOVA; Kruskal–Wallis (KW) one-way ANOVA, KW*–≤0.05, KW**–≤0.01, KW***–≤0.001, Supplementary Table 7).

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Results

TOMAVAC plant development

A binary vector on the pART27 backbone was successfully constructed, inserting the custom synthesized 1,735-bp long recombinant *S1* gene (1,643 bp; Figure 1A), flanking 75-bp 3xFlag-tag (DYKDDDDK-tag), and 24-bp histidine repeats (8xHis; Figure 1B). The *S1* gene sequence information was derived from the consensus sequence of 18 SARS-CoV-2 genotypes, which covered all six mutations (compared to NC_045512.2: G180A, G306T, T476C, G797T, T940C, and G946T) found in both GR and S clade genomes sequenced from Uzbekistan (21). Furthermore, a suspension culture of *A. tumefaciens* strain LBA4404 bearing *pART27::COVID-19_S1* was obtained with a success rate of 96%.

A total of 46 T_0 generation PCR-positive tomato seedlings were recovered out of 405 in-fruit agro-infiltrations, revealing 11% of successful transformation events. Three independent T1 generation tomato transformants (Figure 2A; see Supplementary Figure S1 for original gel image), selected for further analyses, showed stable insertion of the S1 gene in their genomes with relative linear expression levels on average of 0.82–1.63 of the S1 gene in tomato seedlings (Figure 2C; Supplementary Table 1). Tomatoes from single copy transformation event 4, expressing ≈0.77 μg/g S1 protein of SARS-CoV-2, were chosen for further immunization experiments (Supplementary Tables 2, 3).

Immunogenicity and neutralization analyses in mice

We designed a 7-day-spaced, two-dose force-feeding study (Figure 3A). In the TOMAVAC and AstraZeneca (ChAdOx1 NCoV-19; parenteral vaccination control) groups, analysis of the blood serum on day 14 showed insignificant induction of RBD-specific NAbs above 5,300 and 9,000 ng/ml (Figure 3B; Supplementary Table 7) compared to controls. After the second dose of immunization, however, the average titer of RBD-specific NAbs in the TOMAVAC-fed group increased more than 7–9 times (>55,500 ng/ml) in comparison to the controls on day 28 ($p = 0.01$, KW and $p = 0.001$, ANOVA; Supplementary Table 7; Figure 3C).

In the AstraZeneca group, however, the observed NAbs increase (14,669 ng/ml) was insignificant among groups ($p > 0.2$, KW and ANOVA). On day 42, a statistically significant ($p \leq 0.01$, KW and ANOVA) 11- to 14-fold increase (average >43,000 ng/ml) in the titer of RBD-specific NAbs was revealed in the TOMAVAC group (Figure 3D, Supplementary Table 7) and AstraZeneca (average >49,500 ng/ml; $p \leq 0.02$, KW and ANOVA) groups compared to non-vaccinated controls; however, the NAbs increase between the TOMAVAC and AstraZeneca groups was insignificant ($p > 0.8$, KW and ANOVA). Interestingly, relative to day 28, an average 24% decrease in NAbs level was observed in the TOMAVAC group. In contrast, on day 56, the titer of RBD-specific NAbs increased from 15- to 17-fold in the TOMAVAC (average >80,850; $p \leq 0.0001$, ANOVA) and AstraZeneca (average >83,000; $p \leq 0.0001$, ANOVA) groups compared to controls (Figure 3E; Supplementary Table 7).

The analysis of the neutralizing activity of RBD-specific NAbs showed that in the TOMAVAC group, the average neutralization activity was 15% ($p \leq 0.01$, ANOVA or $p \leq 0.05$, KW; Figure 3F) and 25% ($p \leq 0.01$; ANOVA; Figure 3G: Supplementary Table 7) on days 42 and 56, respectively. However, the neutralizing activity of RBD-specific NAbs in the AstraZeneca group was significantly higher (29%−46% on the same periods; $p \leq 0.01$; ANOVA; Figure 3G, Supplementary Table 7) than what was observed in the TOMAVAC group. In the control groups, the level of neutralization was within 5%−7% and was statistically insignificant (Figures 3F, G, Supplementary Table 7). No severe side effects were recorded in mice during or after the consumption of TOMAVAC. Two 90-day-old mice per group, including controls, died at week 8 in the mouse experiment. The cause of death is estimated to be natural, usually occurring with long-period feeding experiments (L.G. Gafurova, Tashkent Pharmaceutical Institute, personal communication).

The analysis of the titer of RBD-specific S-IgA in the TOMAVAC group on day 14 showed a significant induction of 5.9 ng/ml (Figure 4A, Supplementary Table 8) compared to controls. We also detected the induction of RBD-specific S-IgA in the AstraZeneca group, but its level was almost three times lower than that of the TOMAVAC group. After the second dose of vaccination, we revealed a further elevation in S-IgA levels in the TOMAVAC (average 7.5; $p < 0.0001$, ANOVA) and AstraZeneca (average 3.5; $p < 0.0001$, ANOVA) groups compared to controls on day 28 (Supplementary Table 8; Figure 4B). On days 42 and 56, we found a two- to fourfold decrease in RBD-specific S-IgA levels in the TOMAVAC and AstraZeneca groups relative to day 28 (Figures 4C, D).

Concept-of-proof human consumption analysis

In all volunteers consuming TOMAVAC (50 g or ≈38.5 μg S1 protein daily during 3 days), a significant ($p \leq 0.01$, Dunn's multiple comparisons adjusted) positive trend of weekly increase (with an average of +42.28 BAU/ml/3 weeks) of NAbs was observed relative to the initial day levels (Supplementary Figures S4A, C). The increase in serum NAbs started on day 7 with an average of +35.39 BAU/ml ($p \leq 0.01$, Dunn's multiple comparisons adjusted; Supplementary Table 9). NAbs increased on day 14 with an average of +37.73 BAU/ml ($p = 0.06$ in Dunn's multiple comparisons). Furthermore, the statistically significant average +53.44 BAU/ml NAbs increase was detected on day 21 ($p = 0.01$, Dunn's multiple comparisons adjusted; Supplementary Figures S4A, C).

In one participant, a +8.15 BAU/ml increase in NAbs level was determined on day 7; however, on days 14 and 21, a decrease (−11.85 and −8.14 BAU/ml, respectively; Supplementary Table 10) of NAbs was observed relative to the initial level. In another participant, although a positive increase relative to the initial day was recorded on days 7–21, an increased level of NAbs (+13.0 BAU/ml) on day 7 decreased (−8.9 and −5.2 BAU/ml) on days 14 and 21. In contrast, in the control group, a gradual negative trend of weekly decrease (average −28.7 BAU/ml; $p \leq 0.001$, Freidman's ANOVA) was observed in the level of NAbs relative to the initial day (Supplementary Figures S4B, C; Supplementary Table 9). No

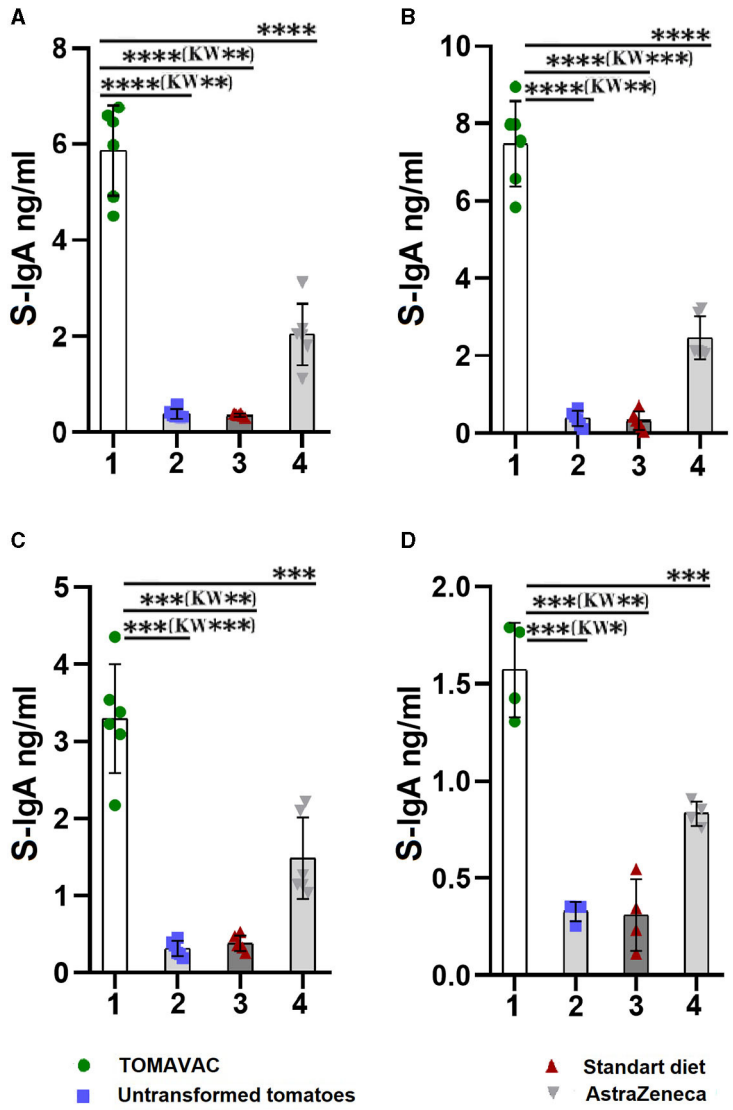

FIGURE 4
Titer of RBD-specific mucosal S-IgA in mice after oral vaccination. **(A)** – titer on day 14 post-vaccination; **(B)** – titer on day 28 post-vaccination; **(C)** – titer on day 42 post-vaccination; **(D)** – titer on day 56 post-vaccination. Statistical significance level (*–≤0.05, **–≤0.01, ***–≤0.001 and ****–≤0.0001) from ordinary one-way ANOVA; Kruskal-Wallis (KW) one-way ANOVA, KW*–≤0.05, KW**–≤0.01, KW***–≤0.001, Supplementary Table 8).

severe side effects were recorded in volunteers during or after the consumption of TOMAVAC. The observed feeling of abdominal pressure on the consumption days was resolved after immunization (Supplementary Table 10).

Discussion

The plant-made COVID-19 vaccine development efforts were recently well-reviewed by Su et al. (20). Many such parenteral candidate vaccines are in their early stages of application, with few commercialized for research and diagnostic purposes (20). In this study, we report the first edible COVID-19 tomato vaccine, TOMAVAC, derived by constitutively overexpressing the consensus sequence of the S1 protein of SARS-CoV-2, covering the sequence variation of SARS-CoV-2 spread in Uzbekistan (26). In contrast, recently, the SARS-CoV-2-S1-Fc fusion protein (16–18) or a SARS-CoV-2 perfusion spice trimer protein (19) was transiently expressed in tobacco and harvested for parenteral vaccination studies in animals.

In our study, among several stable transformation events, we selected the single-copy transgenic genotype with no antibiotic resistance marker gene (event 4), which reduces the chances of gene silencing and instability in the next generations and is the key to the biosafety and purity of the transgenic plant product (27). While our study successfully demonstrated the expression of the S1-specific protein in transgenic tomato plants, we did not observe any discernible phenotypic differences between the transgenic and non-transgenic tomato plants regarding growth, development, or overall appearance. Both sets of plants appeared phenotypically similar and

exhibited normal growth patterns, suggesting that the synthesis of the S1 protein did not visibly affect the external morphology or growth characteristics of the transgenic plants compared to the non-transgenic ones. The fruits of transgenic and non-transgenic plants did not differ in appearance and taste.

TOMAVAC expressing ≈0.77 μg/g S1 protein of SARS-CoV-2 provided an opportunity to design and conduct scalable oral immunization experiments. One explanation for the almost threefold reduced amount of S1 protein in ripened tomatoes compared to the unripened green tomatoes ($p < 0.0001$, ANOVA) could be the influence of the fruit-maturation process. This requires further study and will be crucial for TOMAVAC dose standardization.

The receptor-binding domain (RBD) of the S1 protein was immunogenic and non-allergenic, with very subtle side effects when used as a parenteral protein subunit recombinant vaccine in a previous study (28). Recent studies (16–18) reported that the plant-produced S1 subunit protein vaccines against SARS-CoV-2 were immunogenic without any safety concerns in animal experiments. Previous studies also showed that three injections of 10 μg/dose of RBD-specific antigen of S1 protein adequately induced systemic immunity in mice (29). It was reported that two-dose parenteral vaccination with purified 10 μg and 25–50 μg of tobacco-produced SARS-CoV-2 RBD-Fc fusion protein caused high levels of NAbs in mice and monkeys, respectively (16–18). However, with edible plant-based vaccines, a higher dose of expressed antigen may be needed than that used for parenteral immunizations (13, 14).

Pogrebnyak et al. (30) reported that feeding with 500 mg of lyophilized transgenic tomato fruit (or 50 mg dry roots of transgenic tobacco) expressing 79 kDa SARS-CoV S protein increased fecal IgA levels in mice compared to controls. No increase in serum IgG was found in oral vaccination, although the systemic immune response was caused by a booster dose of S protein injection (30). In contrast, in our study, two courses of spaced weekly feeding of mice with a total of ≈7 g ripened TOMAVAC (≈5.4 μg antigen) bearing intact 61 kDa S1 protein were sufficient for obtaining a primary immune response, which was significantly boosted after the second dose feeding. Contrasting results compared to Pogrebnyak et al. (30) could be due to differences in source, size, sequence, quantity, and oral force-feeding protocol used in our study.

The difference in the increase of NAbs between the TOMAVAC and AstraZeneca groups was insignificant ($p > 0.1$; ANOVA), demonstrating a similar level of induction of systemic immunity via oral vaccination in our study. However, it is noteworthy to mention that due to known evidence of absorptional losses of the oral vaccines in the harsh gastrointestinal environment (13, 14) and to achieve a better NAbs induction correlation between parenteral and oral immunizations in mice, we used over a sixfold diluted dose (16×10^6 infectious units) for AstraZeneca vaccine injection in our study compared to what was used (1×10^8 infectious units) by Silva-Cayetano (31).

The cause of the 24% observed decrease in NAbs level on day 42 (relative to day 28) in the TOMAVAC group is unknown. However, it could be due to calibration differences between sample measurements, as we observed similar higher NAb indices (>5.8) for both negative control groups on day 28 than on day 42 (<3.9). We also found nonsignificant differences in NAbs levels in the TOMAVAC group after data normalization (4.6 on day 28 vs. 4.61 on day 42; Supplementary Table S7).

The neutralization activity of the induced NAbs is pivotal against viral infection (32). Margolin et al. (19) reported that immunization with tobacco-produced SARS-CoV-2 perfusion spike trimer protein was less protective than mammalian cell culture-derived protein in their animal (hamster) model. Our *in vitro* neutralization experiment, based on antibody-mediated blockage of the interaction between the angiotensin-converting enzyme 2 (ACE2) receptor protein and the receptor-binding domain (32), preliminarily suggested an insufficient level of protective response of TOMAVAC against SARS-CoV-2, where we have observed higher neutralization (30%—46%) in parenteral vaccination control with AstraZeneca. This contrasting evidence requires additional studies with TOMAVAC oral vaccination in larger samples and longer durations, which is in progress. There is also a need for an *in vivo* assay of vaccinated animal models and live viruses for a better estimate of vaccine-induced protection against SARS-CoV2 infection.

Mucosal immunity significantly contributes to the defense against viral pathogens by providing a frontline barrier and an effective immune response. Immunization via oral vaccine administration, incorporating specific antigens, stimulates the synthesis of secretory immunoglobulin A (IgA) on mucosal surfaces (33, 34). Studies showed that oral immunization of mice with lactic acid bacteria expressing the SARS-CoV-2 spike (S) protein receptor-binding domain (RBD) S1 subunit increased the fecal IgA levels 1.4-fold higher in the immunized mice than controls after two boosting doses on day 22 (33). In our study, the level of S-IgA in intestinal lavage fluid after two doses of vaccination was 23-fold higher than the standard diet control group on day 28 ($p < 0.0001$; ANOVA). The high S-IgA levels in our study can be explained by higher concentrations of S-IgA within the intestinal mucosa than in feces.

Analysis of the antibody titer on day 56 revealed a decrease in the level of S-IgA in the TOMAVAC group by 3.75-fold compared to day 14 and in the AstraZeneca group by 2.4-fold. However, an increase in the level of S-IgA on days 14 and 28 may indicate the effectiveness of TOMAVAC against the penetration of viruses through the mucous membrane. The tandem-repeat dimeric RBD-based recombinant vaccine was revealed to be immunogenic, protective, and safe in 3-injection (25 μg/dose) clinical trials in humans (27, 35). There is, however, no evidence of edible amounts of S1 antigen that remain intact and adequate for induction of the immune response in the gastrointestinal tract [with 80% acidic and 20% enzymatic digestions (13)] of humans. Previous studies on potatoes reported that three doses of 100 g, containing a total of 1 mg of hepatitis virus B antigen (HBsAg), were partially effective. In contrast, a parenteral vaccination with 40 μg/dose HBsAg caused a sufficient immune response (14). However, there is a caution about administering large quantities of oral vaccine, presumably causing immune tolerance if the consumed antigen molecule does not give sufficient pathogenic signals (13, 14). Here, a 3-day consumption of TOMAVAC, containing ≈38.5 μg [total ≈116 μg vs. 75 μg parenterally used for human vaccination (28)] plant-expressed S1 antigen, was adequate to induce some NAbs in serum

with a positive weekly increasing trend in volunteers vs. a negative weekly decreasing trend in controls (Supplementary Figure S4C). In two volunteers, we observed a decrease in day 7-induced NAbs on days 14 and 21, which remains unclear. This may be due to the personal immune system of volunteers, which requires further study, e.g., parenteral immunization responses in these individuals (36). It also remains unknown whether TOMAVAC protects against currently spread Delta/Omicron variants. However, the results justify future efforts to replace the plant-expressed S1 protein region with emerging VOCs, which are in progress in our laboratory.

Limitations of the study

There are several limitations and demands for future studies, including (a) selection and seed increase of the next generation ($T_{1:5}$) stable self-pollinated genotypes with efficient antigen synthesis in tomatoes (8, 12), (b) evaluation of the stability and duration of storage/shelf life of synthesized S1 protein in mature tomato fruits (8, 14), and (c) study of the influence of gastrointestinal tract condition (13) on tomato-delivered S1 protein to quantify and scale an optimal dosage amount [standardizing of an antigen (14)] for safe consumption of TOMAVAC, inducing efficient immunizations and sufficient protection (19) but not generating immune tolerance (12, 13).

Future studies are needed to investigate possible alterations in biochemical parameters, nutritional composition (such as differences in nutritional content, taste, texture, or other quality aspects), or physiological traits between transgenic and non-transgenic plants to better understand any potential impacts of S1 protein synthesis on plant performance. Future research is also needed, including longitudinal studies with extended follow-up periods, which are essential to comprehensively evaluating the post-vaccination stage effects, the durability of immune responses, and the vaccine's ability to offer sustained protection against COVID-19.

There is an opportunity to develop lyophilized (8, 14) TOMAVAC-based capsules (5) with standardized edible doses for safe delivery and enhanced immunogenicity (14), which demand future investigations with the availability of higher-generation stable transgenic tomato plants. These will help to produce sufficient standardized TOMAVAC for conducting large-scale human consumption clinical trials justifying (a) the efficiency of protection against current and emerging COVID-19 infections and (b) public perception and acceptance (12, 14), as well as proper regulatory measures (20), which require future investigations and investments.

Conclusion

Early evidence from small-scale proof-of-concept study results promises an opportunity for performing inexpensive, eco-friendly, and safe plant-based edible vaccine immunization programs against COVID-19. This might offer longer-term, two-layered protection that limits viral transmission and disease progression, helping to secure global health.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Institutional Ethics Board of the Center of Genomics and Bioinformatics, Uzbekistan. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. The animal study was approved by Institutional Ethics Board of the Center of Genomics and Bioinformatics, Uzbekistan.

Author contributions

ZB: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing. SS: Conceptualization, Investigation, Methodology, Validation, Visualization, Writing – review & editing. DU: Data curation, Investigation, Visualization, Writing – review & editing. MM: Investigation, Methodology, Visualization, Writing – review & editing. KU: Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing. VK: Conceptualization, Investigation, Methodology, Visualization, Writing – review & editing. BR: Investigation, Resources, Writing – review & editing. MA: Data curation, Investigation, Methodology, Visualization, Writing – review & editing. ANA: Methodology, Resources, Visualization, Writing – review & editing. JE: Investigation, Resources, Visualization, Writing – review & editing. BM: Data curation, Investigation, Writing – review & editing. AT: Investigation, Project administration, Resources, Writing – review & editing. AI: Investigation, Validation, Writing – review & editing. TP: Investigation, Writing – review & editing. RR: Methodology, Writing – review & editing. TA: Investigation, Supervision, Writing – review & editing. MM: Investigation, Methodology, Validation, Writing – review & editing. KE: Methodology, Resources, Writing – review & editing. DD: Methodology, Project administration, Writing – review & editing. ST: Project administration, Supervision, Writing – review & editing. AA: Funding acquisition, Supervision, Writing – review & editing. IA: Conceptualization, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The study concept and results have been filed for patenting at the Intellectual Property Agency under the Ministry of Justice of the Republic of Uzbekistan with pending application no. IAP 2022 0247.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1275307/full#supplementary-material

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Unveiling the therapeutic symphony of probiotics, prebiotics, and postbiotics in gut-immune harmony

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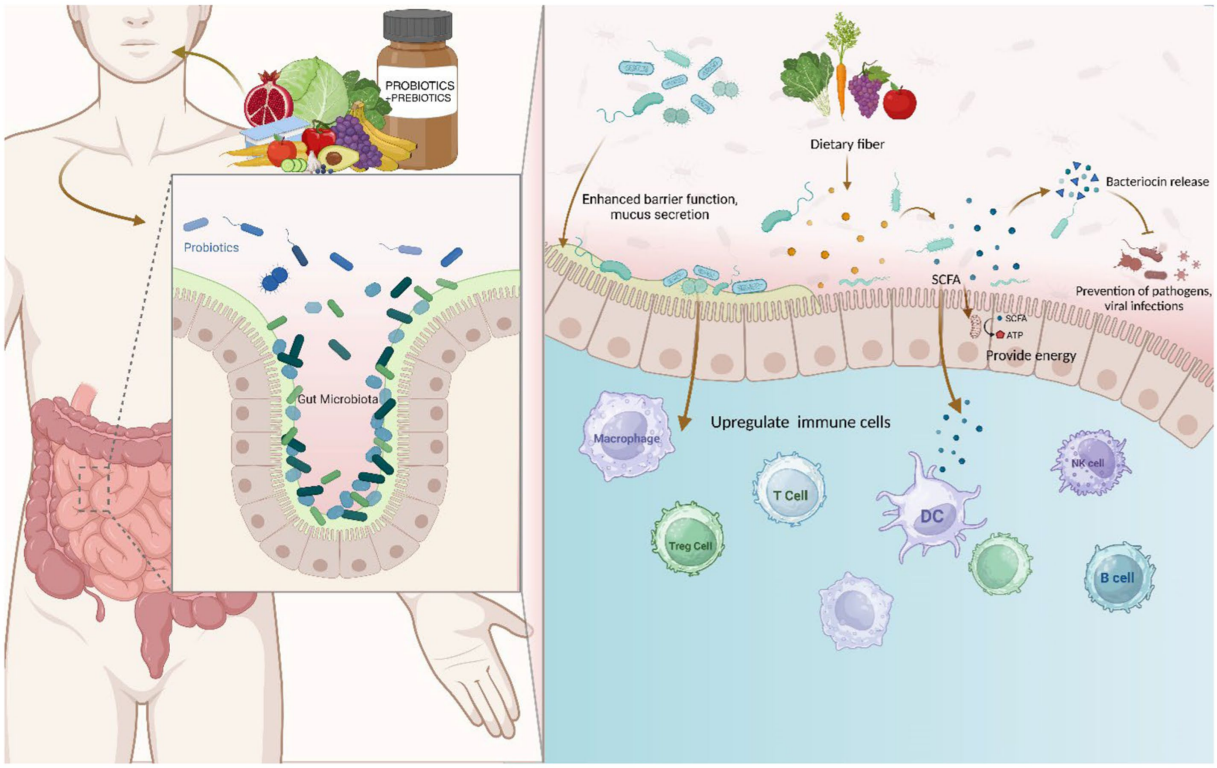
The gut microbiota and immune system interaction play a crucial role in maintaining overall health. Probiotics, prebiotics, and postbiotics have emerged as promising therapeutic approaches to positively influence this complex axis and enhance health outcomes. Probiotics, as live bacteria, promote the growth of immune cells, shape immune responses, and maintain gut barrier integrity. They modify the gut microbiota by fostering beneficial bacteria while suppressing harmful ones. Additionally, probiotics interact with the immune system, increasing immune cell activity and anti-inflammatory cytokine production. Prebiotics, as indigestible fibers, selectively nourish beneficial microorganisms in the gut, enhancing gut microbial diversity and activity. This, in turn, improves gut health and boosts immune responses while controlling inflammation through its immunomodulatory properties. Postbiotics, produced during probiotic fermentation, such as short-chain fatty acids and antimicrobial peptides, positively impact gut health and modulate immune responses. Ensuring quality control and standardization will be essential for successful clinical implementation of these interventions. Overall, understanding and harnessing the gut microbiota-immune system interplay offer promising avenues for improving digestive and immunological health.

KEYWORDS

microbiota, immune system, postbiotics, probiotics, metabolites

Introduction

The term "gut microbiota" refers to the vast and diverse population of microorganisms that reside in the human digestive tract. Trillions of bacteria, viruses, fungi, and other creatures make up this intricate ecology (1). Beyond digestion and food absorption, the gut microbiota plays a pivotal role in supporting general health and well-being. The symbiotic relationship between the gut microbiome and the immune system is crucial (2).



GRAPHICAL ABSTRACT

Regulation of the immune system by gut microbiota

Having a dynamic and bidirectional interaction between the gut microbiota and the immune system is crucial for preserving immunological homeostasis and protecting the body against infections. This communication starts at a young age when microbial populations begin to colonize the gut after birth. The gut microbiota is in constant communication with the immune system, influencing its maturation and performance as (3–6). Early life exposure to a wide variety of gut microbes is crucial for the development of a healthy immune system (7). Beneficial immunological responses to innocuous items (such as food antigens) can be avoided thanks to the presence of gut bacteria, which assist educate immune cells and promote the establishment of immune tolerance. To avoid developing allergies or auto-immune illnesses, this step is essential (8). The gut microbiota communicates with immune cells, including T cells, B cells, and dendritic cells, through various signaling pathways. This communication enhances the immune system's ability to respond to infections while maintaining tolerance for commensal bacteria and dietary antigens. The gut microbiota exerts its regulatory influence on the immune system through various mechanisms. One key mechanism involves the production of metabolites, such as short-chain fatty acids (SCFAs), by certain bacterial species. SCFAs have been shown to modulate immune cell function and promote an anti-inflammatory environment. Additionally, the gut microbiota plays a crucial role in training and educating immune cells, particularly T cells, to appropriately respond to antigens. This process is vital for maintaining immune homeostasis and preventing excessive inflammation. These intricate interactions highlight the dynamic relationship between gut microbiota and the immune system, showcasing the pivotal role of the microbiome in immune regulation (9). The microbiota in the stomach promotes gut health and immunological balance by suppressing inflammation (10). The intestinal barrier is the body's first line of defense against foreign infections and poisons; it is maintained in part by the gut flora. The immune system is better able to monitor what's happening in the stomach when the gut barrier is working properly (11). Emerging evidence suggests that the gut microbiota may influence immune responses beyond the digestive system. This includes impacts on the development of immune cells in the bone marrow and the induction of immunological responses in distant organs, highlighting the need to regulate systemic immune processes.

Immune dysregulation due to an unbalanced microbiota in the gut

Inflammatory Bowel Disease (IBD), allergic manifestations, obesity, and autoimmune pathologies can manifest or intensify due to disruptions in the intricate equilibrium governing the interplay between gut microbiota and the immune system (12). A fundamental facet of human health lies in deciphering the nuanced relationship between the microbiota within the digestive tract and the immune system (13). To improve methods for influencing the composition of gut microbiota and bolstering immune system performance, a thorough comprehension of the interplay between these two systems is essential. Interventions such as probiotics, prebiotics, and

postbiotics are utilized to foster a healthy gut microbiota and strong immune system (Figure 1). Therapeutic therapies targeting the gut-immune axis may become possible as this area of study develops further, opening up exciting new ways to improve human health (14). Importance of maintaining a healthy gut microbiota; (A) The intricate role of the gut microbiota in breaking down complex carbohydrates, proteins, and lipids enhances the digestive process, facilitating the absorption of essential nutrients. This microbial activity optimizes the utilization of vitamins, minerals, and nutrients by the body (15). (B) A diverse and thriving gut microbiota plays a pivotal role in maintaining immunological homeostasis and educating the immune system. This education minimizes the risk of allergic reactions and autoimmune disorders by fostering the body's familiarity with harmless substances (16). (C) Beneficial gut bacteria act as a formidable defense against infections, outcompeting pathogenic microbes for resources and residing space in the digestive tract. A healthy gut microbiota fortifies the immune system, enhancing its ability to combat infections effectively. (D) Synthesis of short-chain fatty acids (SCFAs) by select gut bacteria imparts anti-inflammatory characteristics, contributing to gut health and reducing inflammation. SCFAs offer diverse advantages, including improved metabolic health and diminished inflammation. (E) The gut microbiota establishes a direct communication channel with the brain through the gut-brain axis. For instance, certain gut bacteria have been linked to the production of neurotransmitters like serotonin, which plays a key role in mood stabilization. Additionally, the microbiota's ability to regulate inflammation and immune responses in the gut-brain axis contributes to overall mental well-being. (F) Disturbances in the gut microbiota are associated with disorders of metabolism and obesity. Sustaining a diverse and abundant gut microbiome may contribute to maintaining a healthy weight and metabolic rate. (G) The gut microbiota actively participates in regulating the intestinal barrier, preventing the entry of potentially harmful compounds and mitigating inflammation. A well-functioning intestinal barrier is paramount for immune function

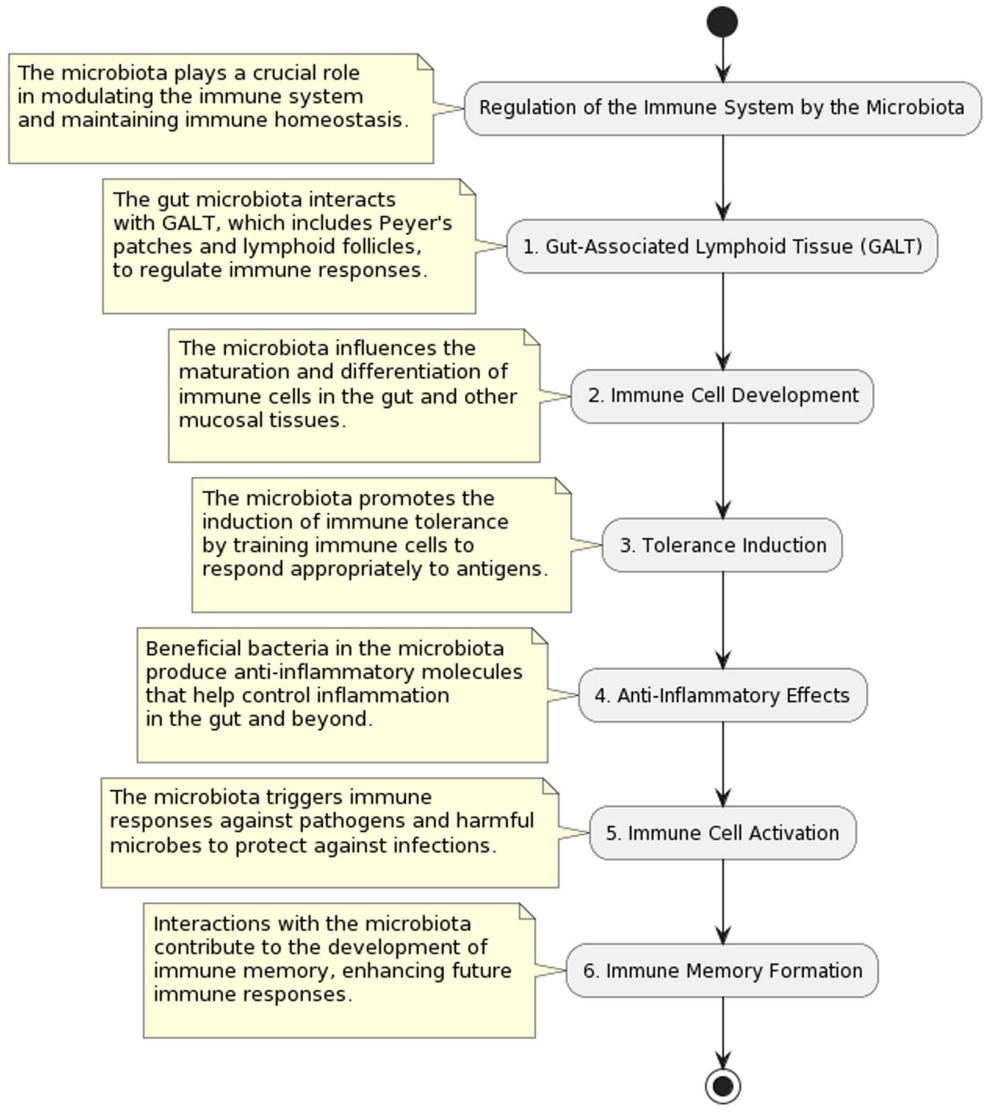


FIGURE 1
Interplay between gut microbiota and the immune system: regulation and implications.

and overall gut health. (H) Dietary fiber undergoes fermentation in the stomach, producing substances beneficial to health. This process, driven by beneficial bacteria, further underscores the importance of a healthy gut microbiota in dietary metabolism. (I) A balanced gut microbiota is pivotal for inflammation regulation. Promoting microbial balance can contribute to reducing systemic inflammation, which is intricately linked to various chronic disorders. The gut microbiota's influence extends across digestion, immunity, metabolism, mental health, and diverse physiological functions. Prioritizing a lifestyle characterized by a balanced diet, regular exercise, and stress reduction promotes a healthy gut microbiome, fostering overall health. Adopting proactive measures, including a balanced lifestyle, and leveraging interventions such as probiotics, prebiotics, and postbiotics, can restore microbial balance and enhance gut health. As research in this field advances, the burgeoning understanding of the gut microbiome's significance opens avenues for novel treatment strategies to elevate human health (17–19).

Mechanisms and immune-boosting effects of probiotics on the gut microbiota

Probiotics, encompassing beneficial bacteria and certain types of yeast, prove advantageous to the host organism when ingested in sufficient quantities. Naturally occurring in fermented foods like yoghurt, kefir, sauerkraut, and kimchi, or available in encapsulated forms, probiotics exhibit crucial features for efficacy (20). Maintaining viability is paramount, often achieved through freeze-drying or encapsulation. While generally considered safe, individuals with compromised immune systems should consult a healthcare professional before probiotic supplement use. Negotiating the acidic stomach environment is a challenge addressed by enteric-coated capsules. Successful probiotics colonize the gut, attaching to the intestinal lining, ensuring prolonged residence and fostering beneficial interactions with the host (21). Their multifaceted benefits include improved digestion, modulation of gut microbiota composition, regulated immune response, reduced inflammation, and protection against infections.

Probiotic action mechanisms

Probiotics orchestrate multifaceted mechanisms influencing the gut microbiota and contributing to host well-being. Firstly, they modulate the composition and diversity of the gut microbiota, actively maintaining a harmonious microbial balance by outcompeting pathogenic microbes for nutritional resources and habitation sites. Secondly, probiotics exhibit a pivotal role in stimulating the immunological system. Through intricate interactions with dendritic cells and T cells in the gut-associated lymphoid tissue, probiotics play a pivotal role in activating and modulating immunological responses. This activation initiates a cascade of events, including the release of cytokines, which serves to suppress inflammation-inducing factors while concurrently enhancing those with anti-inflammatory properties. These immunomodulatory effects contribute to the overall immune-boosting properties of probiotics. Thirdly, probiotics play a crucial role in fortifying the integrity of the gut epithelial barrier. By reducing permeability, they enhance the barrier's resistance to hazardous chemicals and potential infections, thereby contributing to overall gut health. Fourthly, the production of bioactive compounds, specifically short-chain fatty acids (SCFAs), stands out as a significant action of probiotics. SCFAs, synthesized by probiotics, exert anti-inflammatory effects and confer various health benefits. Finally, probiotics showcase their preventive prowess by directly inhibiting the development and activity of pathogenic bacteria and viruses in the digestive tract. This inhibition contributes to illness prevention and underscores the proactive role of probiotics in maintaining optimal health. Described as "live microorganisms with well-defined characteristics," probiotics emerge as versatile agents enhancing host health through their positive influence on the digestive system, immune responses, and overall well-being. Integrating probiotics into a balanced diet and active lifestyle proves instrumental in supporting gut health and fine-tuning the intricate balance between beneficial and harmful bacteria in the gastrointestinal tract, thereby offering a holistic approach to health optimization (18, 19, 22). Probiotics play a vital role in cultivating a healthy and diverse gut microbiota, as depicted in Figure 2. Through various mechanisms, they influence the composition and activity of gut microbes. Probiotics engage in competitive exclusion, out-competing pathogenic microbes for adhesion sites and nutrients to foster a balanced microbiota. Certain bacteria in probiotics regulate stomach acidity, creating an inhospitable environment for harmful germs (23). Additionally, probiotics produce antimicrobial compounds, stimulate mucus production, and modulate the immune system, enhancing anti-inflammatory responses. Some probiotics operate akin to prebiotics, fostering the growth of beneficial bacteria, while cross-feeding interactions influence the metabolic activity of other microbes. Probiotics also regulate gut motility, affecting the spread of bacteria in different gut regions. Strain-specific effects, individual responses, and the duration of probiotic treatment influence the extent of these alterations. In essence, probiotics' ability to shape gut microbial composition is pivotal for promoting gut health, immunity, and overall well-being, with ongoing research pointing toward personalized probiotic therapies for specific cases of gut dysbiosis (24). Modulating immunological responses and enhancing immune cell activity constitute pivotal roles played by probiotics. Predominantly impacting the gut-associated lymphoid tissue (GALT) and the gut epithelium, where a significant portion of immune cells resides, probiotics exert profound influence on the immune system. Key mechanisms through which probiotics shape immunological responses and immune cell activity include.

Regulation of innate immune cells

Probiotics adeptly regulate innate immune cells like macrophages, dendritic cells, and natural killer (NK) cells. This regulation enhances the antimicrobial activity of these cells by stimulating the production of cytokines, notably interleukin-12 (IL-12) and interferon-gamma (IFN-).

Modulation of adaptive immunity

Demonstrating impact on adaptive immunity, probiotics influence the activity of T cells and B cells. They foster the development of

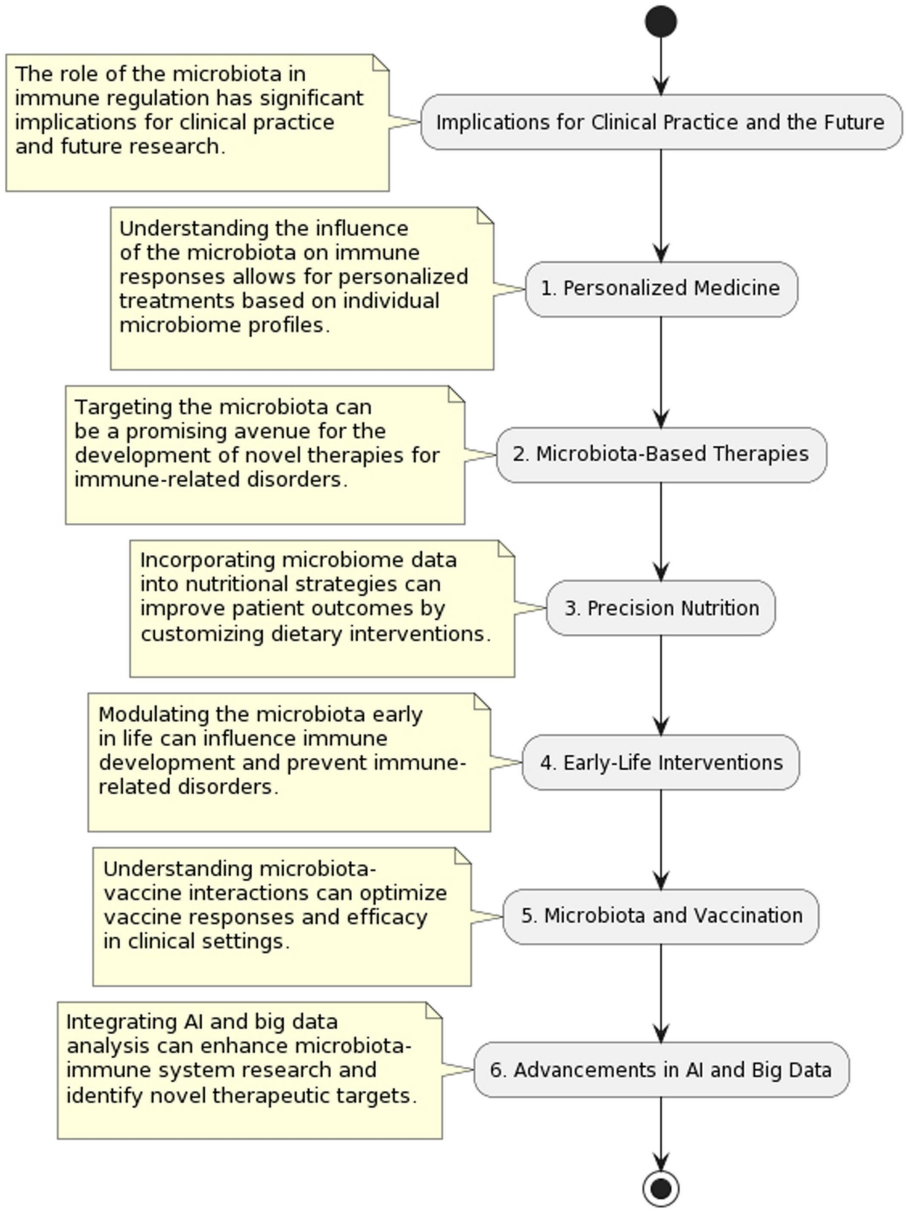


FIGURE 2
Immune system's impact on gut metabolism: regulation and significance.

regulatory T cells (Tregs), pivotal for limiting immunological responses. Additionally, probiotics boost mucosal immune responses, encouraging B cells to produce more immunoglobulin A (IgA) antibodies.

Anti-inflammatory effects

Probiotics exhibit anti-inflammatory effects by suppressing the production of inflammatory cytokines, including tumor necrosis factor-alpha (TNF-) and interleukin-6 (IL-6). This anti-inflammatory action enhances immunological homeostasis, mitigating the risk of inflammatory disorders.

Preservation of gut epithelium barrier

Recognizing the crucial role of the gut epithelium barrier, probiotics contribute to maintaining its integrity. A properly functioning intestinal barrier reduces the workload on the immune system, effectively preventing inflammation.

Induction of immunological tolerance

Probiotics facilitate the development of immunological tolerance, aiding the immune system in distinguishing between harmless and hazardous chemicals. This preventive measure reduces the likelihood

of inappropriate immune responses to innocuous antigens and promotes the formation of regulatory immune cells.

Enhancement of phagocytic activity

Probiotics have been observed to increase the phagocytic activity of immune cells, particularly macrophages. This enhancement empowers immune cells to efficiently engulf and destroy invading pathogens.

Antiviral action

Certain probiotics exhibit antiviral properties by reducing virus replication and influencing the body's innate antiviral defenses.

Systemic impact via gut microbiota

The favorable effects of probiotics on gut microbiota have systemic repercussions, influencing immunological responses throughout the body. The intimate connection between gut health and overall immune function underscores the broad-reaching impact of probiotics on human health. (25–30).

Anti-inflammatory, immune-boosting, and response-boosting effects may all be possible thanks to probiotics. Allergies, autoimmune illnesses, and inflammatory bowel disorders are examples of situations in which immunological dysregulation plays a significant role, making these effects all the more important. It's worth noting, too, that different probiotic strains and individuals' preexisting immunological statuses may produce different degrees of immune-modulatory effects from probiotics. The use of probiotics as adjuvants to improve immunological function and responsiveness in a variety of therapeutic contexts is gaining popularity as research in this field continues to advance (31–33).

Beneficial gut bacteria and immunomodulation

Prebiotics are a type of indigestible fiber or substance that provides nutrition for Beneficial gut bacteria in the digestive tract. Prebiotics are not living organisms, unlike probiotics which are active microbes. Instead, they are a functional diet that increases the population and activity of Beneficial gut bacteria in the digestive tract: Beneficial gut bacteria and the role of prebiotics in encouraging their growth: Prebiotics, resistant to digestion in the upper gastrointestinal system, reach the colon undigested, becoming substrates for fermentation by beneficial microbes such as Lactobacilli and Bifidobacterial. This fermentation yields short-chain fatty acids (SCFAs) like acetate, propionate, and butyrate. Colonocytes lining the colon heavily rely on SCFAs for energy, crucial for maintaining intestinal integrity. Additionally, the acidified gut environment resulting from SCFA generation inhibits the growth of harmful bacteria, fostering the dominance of beneficial ones. Regular consumption of prebiotics modulates the gut microbiome, promoting stability and diversity. Notably, prebiotics, particularly inulin and fructo-oligosaccharides (FOS), selectively stimulate the growth of Bifidobacterial and Lactobacilli, recognized as beneficial bacteria with various health-promoting properties. This intricate interplay underscores the pivotal role of prebiotics in shaping a resilient and balanced gut ecosystem. Prebiotics and Immune Modulation: Prebiotics have immunomodulatory effects in addition to their role in supporting the development of beneficial gut flora. The gut microbiota's interaction with the gut-associated lymphoid tissue (GALT) and the host immune system is a major mediator of these effects. Prebiotics have several important impacts on the immune system, including (19, 21, 34).

1 Increased Mucosal Immunity: Prebiotics have been shown to increase mucosal immunity by increasing IgA antibody production. When it comes to keeping the gut mucosa safe from dangerous bacteria and toxins, IgA is a key player.
2 In the gut-associated lymphoid tissue, prebiotics can regulate the function of immune cells including T cells and dendritic cells. They aid in keeping the immune system in check and avoiding overactivation.
3 Effects on Inflammation Prebiotics improve intestinal barrier function and encourage a more balanced immune response by encouraging the development of beneficial bacteria.
4 Influence on Generalized Immune Responses Due to the strong relationship between the stomach and the immune system. Systemic impacts on immune responses may result from prebiotics' ability to alter the makeup of gut microbiota.

Prebiotics are important because they help keep the digestive tract and immune system healthy. Consuming prebiotic-rich foods regularly has been shown to improve gut microbiota and general health. These foods include garlic, onions, bananas, asparagus, and whole grains. Prebiotics are becoming increasingly popular as a dietary approach to improve gut health and immune function as research in this field continues to advance.

Key effects of prebiotics in modulating the immune system:

1 Involvement of gut-associated lymphoid tissue (GALT) in immune system development and maturation is regulated by prebiotics, which promotes the growth of beneficial gut bacteria like Bifidobacterial and Lactobacilli. Peyer's patches and lymphoid follicles are examples of GALT structures that play a crucial role in immune monitoring and the initiation of immunological responses.
2 Dendritic cells, macrophages, and T cells, all members of the immune system, can have their activity in the GALT modulated by prebiotics. Interleukin-10 (IL-10) and other anti-inflammatory cytokines are produced in response, which aids in controlling immune responses and limiting inflammation.
3 Prebiotics promote mucosal immunity by promoting the development of immunoglobulin A (IgA) antibodies in the intestine. IgA is essential because it prevents infections and toxins from damaging the gut mucosa by binding to them.
4 Prebiotics assist decrease intestinal inflammation by increasing the population of beneficial bacteria and strengthening the intestinal barrier. Gut inflammation and immunological reactions can be avoided with a healthy gut microbiota and a well-functioning gut barrier.

5 Because of the intimate relationship between the gut and the immune system, prebiotics' ability to alter the composition of the gut microbiota can have far-reaching consequences for immune function. This has the potential to result in a more stable and controlled immunological response.
6 Autoimmune illnesses, metabolic problems, and gastrointestinal issues are only some of the diseases and ailments that may be avoided by avoiding chronic inflammation (35, 36).

By encouraging a gut environment less favorable to the growth of pro-inflammatory bacteria, prebiotics can aid in the prevention of chronic inflammation.

Bioactive by-products of probiotics (postbiotics)

Postbiotics are the bioactive chemicals or metabolites that probiotic bacteria create following fermentation or interaction with the gut microbiota. Postbiotics are the metabolic by-products of probiotic activity, as opposed to probiotics, which are living microorganisms, and prebiotics, which are non-digestible fibers that encourage the growth of good gut bacteria. These bioactive substances improve the health of the host in several ways. Due to their wide range of biological activity, postbiotics have recently garnered interest as possible therapeutic agents (35).

A Few Postbiotic Examples:

1 One of the most well-known and well-investigated postbiotics is short-chain fatty acids (SCFAs). Beneficial bacteria, such as Bifidobacterial and Lactobacilli, ferment prebiotic fiber and dietary fiber to generate probiotics. Acetic acid, propionate, and butyrate are the most common SCFAs. SCFAs are essential for gut health because they fuel colonocytes, improve the integrity of the gut barrier, and control immunological responses. In addition to their anti-inflammatory qualities, they also help prevent gastrointestinal problems and several types of metabolic ailments.
2 Certain probiotic bacteria, such as Lactobacillus and Bifidobacterium species, generate bacteriocins, which are antimicrobial peptides. Inhibiting the growth of pathogenic microbes and helping to keep a healthy gut microbiota are two of the many benefits of these peptides' antimicrobial action.
3 Complex carbohydrates known as exopolysaccharides (EPS) are produced by probiotic bacteria. They operate similarly to prebiotics and can promote the development of good gut flora. EPS can improve the gut's barrier function and also have immunomodulatory effects.
4 Fermentation of carbohydrates by probiotic bacteria results in the production of organic acids including lactic acid and acetic acid. These organic acids help maintain an acidic gut environment, which discourages the development of harmful bacteria while encouraging the expansion of helpful ones.
5 Among the many components of bacterial cell walls are peptidoglycans, the breakdown products of which can have immunomodulatory effects on the host immune system.
6 Secretory factors: Probiotic bacteria can impact host immunological responses, gut barrier function, and other physiological processes by the secretion of a wide variety of bioactive chemicals and molecules (36–40).

There has been a lot of discussion about postbiotics recently in the context of therapeutic therapies for gut health. They have the potential to be superior to probiotics in several ways, including being more stable, easily stored, and administered, and requiring no active microbes. More study is required, however, before postbiotics can be effectively used to treat a wide range of health issues. With their wide range of biological effects, postbiotics help maintain healthy gut microbiota and keep the immune system in check. Probiotic bacteria create these bioactive compounds during fermentation or in response to the gut microbiota and environment. Consider the following effects of postbiotics on the gut microbiota and immune regulation Postbiotics like short-chain fatty acids (SCFAs) and exopolysaccharides (EPS) can provide food for beneficial bacteria.

1 In the gut, such as Lactobacilli and Bifidobacterial. Postbiotics boost the development and activity of these bacteria by providing them with favorable habitats and nutrients, increasing their dominance in the gut microbiota. This increases the diversity and stability of the microbiome in the digestive tract, which is beneficial to both digestive health and general health.
2 Some postbiotics, such as bacteriocins and organic acids, have antibacterial capabilities that can impede the development and spread of dangerous pathogenic microorganisms in the digestive tract. Postbiotics aid in keeping the gut healthy by decreasing the population of harmful microbes, which helps to ward against infections and disorders that originate in the digestive tract. Postbiotics, such as SCFAs and EPS, have been demonstrated to improve intestinal permeability. To prevent dangerous compounds and germs from crossing from the gut lumen into circulation, they can fortify the tight junctions between intestinal epithelial cells. To prevent inflammation and keep the immune system balanced, a strong intestinal barrier is necessary.
3 The immune system can be modulated by postbiotics because of their direct interaction with immune cells in the gut-associated lymphoid tissue (GALT). SCFAs, for instance, might boost the production of anti-inflammatory cytokines like interleukin-10 (IL-10) and encourage the formation of regulatory T cells (Tregs). This results in a more normal immune response, which in turn decreases inflammation and protects against autoimmune diseases.
4 Tolerance Induction: Postbiotics can help the immune system learn to distinguish between innocuous and hazardous chemicals, a process known as immunological tolerance. Postbiotics aid in preventing inappropriate immune responses to innocuous antigens and allergens by instructing the immune system and encouraging the growth of regulatory immune cells.
5 Many postbiotics, including short-chain fatty acids (SCFAs) and peptidoglycans, have anti-inflammatory effects. They can regulate the activity of inflammatory immune cells and reduce the production of inflammatory cytokines. This anti-inflammatory impact is critical for protecting against chronic

inflammation and keeping the gut in good working order. (29, 30, 36, 38–40).

By increasing the population of helpful gut bacteria while decreasing the population of pathogenic bacteria, bolstering the function of the gut barrier, and regulating immunological responses, postbiotics help maintain a healthy gut microbiota and regulate the immune system. Potential advantages for gut health, immunological function, and general well-being have been linked to the use of postbiotics in the form of probiotic-rich meals or postbiotic supplements. The exact mechanisms of action and the ideal doses for obtaining the intended health results require more study (21).

Gut-immune system modulation for targeted therapeutic interventions

In the last several years, there has been a lot of research into immune-related illnesses, metabolic problems, and gastrointestinal issues. These bioactive compounds have therapeutic potential for improving health outcomes in a variety of particular health disorders by influencing gut microbiota and the immune system (29–31, 39, 40).

1 Digestive Problems
   a Bloating, stomach discomfort, and constipation are all signs of irritable bowel syndrome (IBS), and probiotics have been investigated for their ability to alleviate these conditions. Inflammation can be reduced and gut barrier function can be restored in IBS patients by taking certain probiotic strains like *Bifidobacterium infantis* and *Lactobacillus plantarum*.
   b Both probiotics and postbiotics have demonstrated positive results in the treatment of inflammatory bowel disease (IBD). Disease severity and remission rates in people with ulcerative colitis and Crohn's disease have been linked to the use of certain probiotics, such as Saccharomyces boulardii and *Bifidobacterium breve*. The anti-inflammatory effects of postbiotics, especially short-chain fatty acids, are important in the regulation of inflammatory bowel disease (IBD) symptoms.
2 Disorders of Metabolism
   a Inulin and oligofructose are two examples of prebiotics that have been investigated for their possible role in alleviating obesity and its associated metabolic abnormalities. In turn, the SCFAs produced by these Beneficial gut bacteria affect energy metabolism and adipose tissue function. Weight loss and enhancement of metabolic indicators in obese people have also been linked to the use of probiotics such as *Lactobacillus rhamnosus* and *Bifidobacterium lactis*.
   b Glycemic management and insulin sensitivity in people with type 2 diabetes have been studied as a possible benefit of probiotics and prebiotics. These bioactive compounds improve glucose metabolism and decrease inflammation in diabetes patients by altering the makeup and function of the gut microbiota.
3 Disorders of the immune system
   a It has been investigated whether or not probiotics and prebiotics can mitigate allergy diseases including eczema and rhinitis. They are essential in the regulation of the balance between Th1 and Th2 immune responses, which are responsible for the development of allergic disorders.
   b Evidence is mounting that probiotics and prebiotics may be useful in controlling autoimmune illnesses including rheumatoid arthritis and multiple sclerosis by altering the composition of the gut flora. These bioactive compounds affect immunological dysregulation and might reduce autoimmune reactions.
4 Disabilities of the Mind

New evidence suggests the gut-brain axis is important for psychological well-being. The potential of probiotics and prebiotics to boost mood and alleviate stress and depression has been studied. These bioactive chemicals have the potential to alter the gut microbiota and increase the production of neuroactive molecules, both of which have the potential to improve mental health.

Individual differences in gut microbiota composition and immune responses must be taken into account, as must the kind and quantity of bioactive compounds like probiotics, prebiotics, and postbiotics when they are used to treat specific health disorders. To further understand the mechanisms of action and improve their usage for therapeutic therapies targeting certain disorders, more research and well-planned clinical trials are required.

Insights into the mechanisms of the gut-brain connection

The gut-brain axis, a complex communication network involving the gastrointestinal tract, its microbiota, and the central nervous system, plays a pivotal role in influencing various physiological processes and health outcomes. Trillions of microorganisms in the gut microbiota produce neurotransmitters, short-chain fatty acids, and other bioactive compounds that impact central nervous system and brain function, influencing mood, emotions, and cognitive performance (1). The gut-brain axis relies on intricate signaling pathways, including neurological, immunological, and endocrine mechanisms, with the vagus nerve serving as a crucial conduit for bidirectional communication between the digestive tract and the central nervous system (2). The gut-associated lymphoid tissue (GALT), a significant component of the immune system in the intestinal lining, interacts with gut bacteria and modulates immune responses, affecting brain function either directly or indirectly through systemic inflammation (3). Dysregulations in the gut-brain axis have been associated with stress reactions, mood disorders, cognitive performance alterations, and various diseases such as gastrointestinal, metabolic, and neurodegenerative conditions (4). Understanding the implications of the gut-brain axis has opened avenues for potential interventions, including probiotics, prebiotics, and postbiotics, offering novel strategies for enhancing mental health (5). Lifestyle factors like dietary choices and stress management also influence the gut-brain axis, providing additional avenues for promoting brain health. In conclusion, the dynamic interplay between the gut microbiota, immune system, and the brain within the gut-brain axis underscores its significance in health and disease, offering valuable insights for innovative treatments and holistic health improvement (19, 32, 41–43).

Positive effects on mental and neurological health due to probiotics, prebiotics, and postbiotics

Psychobiotics, encompassing probiotics, prebiotics, and postbiotics, have emerged as promising agents that may influence mental health and wellness through the intricate gut-brain axis. Supported by a body of studies (19, 32, 41–43), psychobiotics have been shown to modulate the gut microbiota, stimulate the development of beneficial bacteria, and alleviate conditions linked to gut dysbiosis, such as depression and anxiety. Through the synthesis of neurotransmitters like serotonin, gamma-aminobutyric acid, and dopamine, psychobiotics contribute to mood regulation and stress response, potentially alleviating depressive and anxious feelings. Furthermore, their interaction with the immune system helps prevent neuroinflammation and neurodegenerative disorders. Psychobiotics impact the hypothalamic–pituitary–adrenal (HPA) axis, reducing stress hormone levels and promoting mental well-being. Through the gut-brain axis, they send signals that influence brain activity, mental performance, and emotional reactions. Psychobiotics may enhance synaptic plasticity, neurotrophic factor production, and maintain the blood–brain barrier's integrity, positively impacting learning, memory retention, and brain health. Additionally, they exhibit anti-inflammatory effects, potentially guarding against neurodegenerative diseases and mood disorders linked to neuroinflammation. While further research is needed to fully understand their mechanisms and individual responses, psychobiotics hold promise as supportive or adjunctive therapies for various mental health issues, reflecting their potential to contribute to both brain health and emotional stability (41–43).

Implications for clinical practice and the future

The potential therapeutic uses of probiotics, prebiotics, and postbiotics have garnered a lot of attention in the healthcare profession. Studies on the positive benefits of these bioactive compounds on gut health, immunological function, and general well-being have shown promise in a variety of clinical contexts (Figure 3). Listed below are some of today's most promising clinical uses of probiotics, prebiotics, and postbiotics in medicine (36–40):

1 Digestive Problems

Irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), and infectious diarrhea are all gastrointestinal conditions for which probiotics have been the subject of substantial research. They have been shown to alleviate symptoms and enhance gut health by restoring the natural balance of gut bacteria, decreasing inflammation, and strengthening the gut barrier.

2 Help Your Immune System

The potential immunomodulatory effects of probiotics and prebiotics have been studied. Increased immune cell activity, increased synthesis of anti-inflammatory cytokines, and strengthened immunological responses are all possible results of their use. Therefore, they may aid in immune system development and decrease the likelihood of infection.

3 Wellness for Women

Vaginal infections including bacterial vaginosis and yeast infections are common among women, and probiotics have shown promise in preventing and treating these conditions. Probiotics can keep your vagina healthy and cut down on the frequency of infections by reestablishing the natural balance of bacteria there.

4 Diseases of the immune system

There has been research on the potential of probiotics for allergy prevention and management, especially in young children. Inhibiting allergy reactions by modulating immunological responses and fostering immune tolerance may be one of their effects.

5 Concerning the Mind

Probiotics and prebiotics are being studied for their potential to improve mental health issues like depression, anxiety, and stress. They may create neuroactive chemicals that affect mood and emotional well-being, and hence modify the gut-brain axis.

Future possibilities and possible improvements

To treat particular health disorders and precisely control the gut microbiota, microbiome-based medicines, such as genetically modified probiotics and postbiotics, may be developed in the future, thanks to recent developments in the field of microbiome research. Research into postbiotics and the bioactive metabolites they produce may lead to novel therapeutic uses and offer viable alternatives to live probiotics. It's possible that postbiotics might be more suited for pharmaceutical formulations due to their increased stability and shelf life. Profiling the microbiota in a person's gut using cutting-edge technology may allow for more in-depth and precise evaluations of gut microbiota composition. This will allow for the development of probiotic and prebiotic therapies that are uniquely suited to each person's microbiome (43, 44).

The gut microbiota is extremely personalized, and people's reactions to probiotics, prebiotics, and postbiotics can vary widely.

1 In-depth profiling and analysis may be necessary to determine which actions will be most beneficial for each individual.
2 Despite widespread assurances of the safety of probiotics, prebiotics, and postbiotics, some people, particularly those with weakened immune systems or preexisting diseases, may be at risk. It is essential to ensure that tailored therapies are safe and well tolerated.
3 The genuine efficacy and sustainability of probiotics, prebiotics, and postbiotics in personalized medicine require a thorough evaluation of their long-term impact on health outcomes.
4 There is the problem of quality assurance and regulation, which arises since tailored probiotic and prebiotic therapies may be classified as medications or medical devices. It is crucial to do thorough quality checks, adhere to industry standards, and conduct risk assessments.
5 Cost and Availability: There are ethical concerns about fair access to such therapies if the widespread implementation of tailored probiotic and prebiotic therapy is prohibitively expensive.
6 Data Privacy and Security: Protecting individuals' personal information is essential while collecting and analyzing (45–53) massive volumes of microbiome data for targeted

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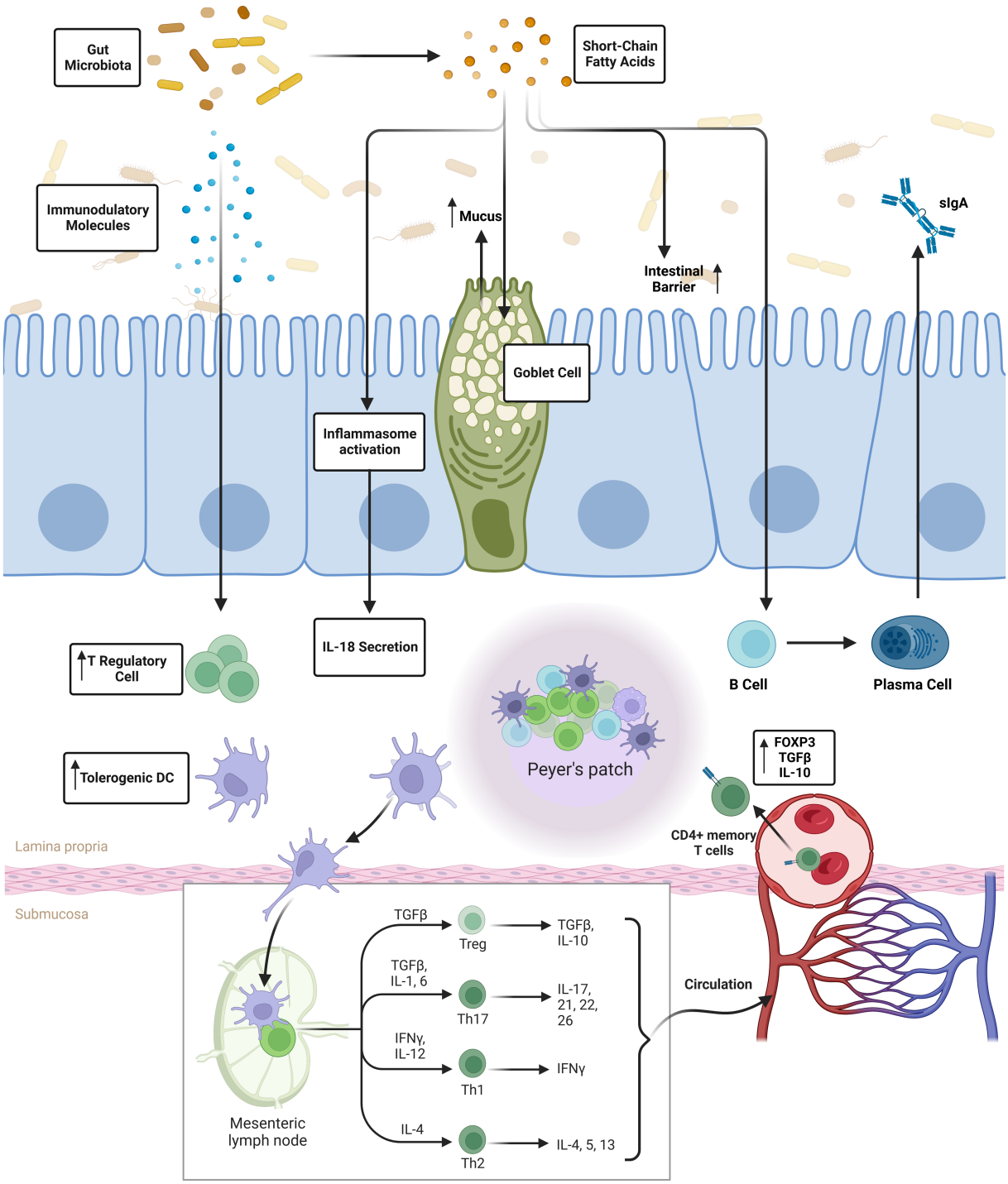

FIGURE 3
Microbiota in clinical practice: implications and applications.

therapies. In conclusion, the discipline of personalized medicine shows significant potential for enhancing health outcomes and disease management based on an individual's unique gut microbiome composition through the use of probiotics, prebiotics, and postbiotics. To fully realize the potential of these treatments for customized healthcare, however, it is necessary to solve obstacles relating to individual variability, safety, efficacy, regulatory concerns, and accessibility. It will be essential to continue research, develop new technologies, and foster cooperation among academics, healthcare providers, and policymakers to move the field forward and transform microbiome-based therapies into feasible and successful personalized medicine methods.

Conclusion

Potential therapeutic advantages in a wide range of health disorders may be attained by the use of probiotics, prebiotics, and postbiotics, all of which modulate the gut microbiota-immune system axis. New possibilities for personalized therapy and therapies based on the microbiome have emerged as a result of our better knowledge of this complex interaction. As it affects immune function, nutritional absorption, metabolism, and even mental health, a healthy gut microbiota must be maintained for maximum health. Beneficial probiotic bacteria can alter the makeup of gut microbes and help keep the immune system in check. On the other side, prebiotics feed Beneficial gut bacteria in the stomach, encouraging their proliferation and activity. Beneficial benefits on gut health and immunological function have also been shown for postbiotics, which are bioactive molecules produced from probiotics. Probiotics have demonstrated positive results in treating a variety of diseases and illnesses, including those affecting the digestive system, the immune system, the nervous system, and the metabolism. They have therapeutic promise due to their capacity to regulate immunological responses and promote healthy gut barrier function. Research into prebiotics has focused on their potential to modulate the immune system, to improve gut health and immunological function. The study of how changes in the gut microbiota affect the immune system is an exciting new topic with plenty of potentials. The individual variability, safety, efficacy, and cost-effectiveness of probiotics, prebiotics, and postbiotics used in a tailored manner must be carefully considered. Integrating interventions into clinical practice relies heavily on ensuring their quality is controlled and standardized. Future innovations may involve the creation of synthetic prebiotics and targeted probiotics that are specifically designed for an individual's gut microbiota. The accuracy of tailored therapies will increase as technology improves microbiome profiling methods. The probiotics, prebiotics, and postbiotics' ability to alter the composition of gut microbiota and the immune system hold great promise as a new medical frontier. Personalized medicine techniques based on gut microbiome analysis may transform disease prevention, treatment, and general health optimization as research in this field continues to improve. A new age of individualized healthcare that prioritizes individual well-being and enhances health outcomes for varied populations may be ushered in by adopting a holistic approach to gut health and immune function.

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Food-specific IgG4-guided diet elimination improves allergy symptoms in children

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Allergic diseases in children are major public health concerns due to their widespread and rising prevalence. Food-specific immunoglobulin G4(FS-IgG4) has been detected in patients with allergic diseases, but its clinical significance is still debated. In the present study, 407 children with allergic diseases were recruited and categorized into three groups according to the different systems involved: the respiratory system group, the skin system group, and a multiple system group, with the collection of clinical symptoms and serum antibodies, including total immunoglobulin E (IgE), house dust mite (HDM) IgE, food-specific IgE (FS-IgE), and FS-IgG4. Part of these patients were followed up with the intervention of FS-IgG4-guided diet elimination with or without add-on probiotics supplement. The analysis at baseline revealed distinct serum levels of different antibodies. The positive rate of FS-IgG4 in all groups was more than 80%, and the proportion of total IgE and FS-IgG4 both positive in the multi-system group was the highest (p=0.039). Egg and milk were the foods with the highest positive rate of FS-IgG4 in all groups. After diet elimination for more than 3 months, serum FS-IgG4 in children significantly decreased (P<0.05) along with the improvement of clinical symptoms, regardless of the add-on of probiotics. However, the intervention did not impact the serum levels of total IgE, FS-IgE, and HDM IgE. There was no further decrease of serum FS-IgG4 level in children followed up for more than 1 year, which may be related to noncompliance with diet elimination. Multivariate regression analysis revealed that the decline of serum FS-IgG4 was an independent predictable factor for the improvement of clinical symptoms (adjusted OR:1.412,95%CI 1.017–1.96, p=0.039). The add-on of probiotics showed less efficiency in reducing the FS-IgG4 level in more patients with relief of clinical symptoms. Our results confirmed the correlation between FS-IgG4 and allergic diseases, and the decreased FS-IgG4 could be a useful predictor for the improvement of allergic symptoms. FS-IgG4-guided diet elimination is an efficient treatment for allergic diseases. Our study adds solid data to the clinical significance of FS-IgG4 in allergic diseases.

KEYWORDS

food-specific IgG4, children, allergy, diet elimination, IgE

Introduction

Allergic diseases have become some of the most prevalent chronic conditions affecting children worldwide. The prevalence of allergic diseases in children has increased dramatically over the past thirty years, as evidenced by three-phase surveys conducted by the International Study of Asthma and Allergies in Childhood (ISAAC) (1, 2). Pediatric allergies in China have also become a rising burden alongside rapid economic development and urbanization (3). Consequently, the prevention and intervention of pediatric allergies present a significant challenge for clinical practitioners. Management of allergic diseases extends beyond medication, with considerations such as food management proving to be crucial.

In the typical atopic march, atopic dermatitis (AD) often serves as the initial manifestation of allergy in infancy, followed by staggered occurrences of food allergy, allergic rhinitis, and allergic asthma (4). Food allergy is defined as an adverse immune response to food proteins and represents a spectrum of clinicopathologic manifestations, including gastrointestinal disturbances, hives, eczema, and airway inflammation, ranging in severity from mild to life-threatening (5). It can be categorized into three types: immunoglobulin E (IgE) mediated, non-IgE mediated, or mixed (6). IgE-mediated food allergy is typically characterized by exposure to very small amounts of allergic foods triggering clinical symptoms within minutes to hours after ingestion (5, 7). In contrast, non-IgE mediated food allergy has a delayed onset of symptoms, often presenting chronically, making the association with the specific allergens obscure and challenging to diagnose (7). Manifestations primarily include skin reactions (such as AD, contact dermatitis, and herpetiformis), respiratory reactions (such as Heinner syndrome), or gastrointestinal reactions such as eosinophilic esophagitis (EOE) (8). Mixed food allergies involve both IgE-dependent and IgE-independent pathways.

Apparently, IgE-mediated food allergies are widely recognized and feared by those affected. It is generally accepted that food can induce various forms of allergic reactions, from urticaria to asthma and, in urgent conditions, anaphylaxis, through specific IgE-mediated mast cell degranulation. Therefore, food-specific IgE (FS-IgE) is conventionally used as a clinical screening test for food allergy or food-related reactions. In fact, mast cell degranulation can also be activated via immunoglobulin G (IgG). Apart from FcεRI, mast cells and basophils in humans and mice also express Fcγ receptors (FcγRs) that bind to IgG antibodies. These IgG antibodies have been shown to activate mast cells even before the discovery of IgE (9). Compared to IgE, IgG antibodies are more complex in structure and biology, and they have four subclasses: IgG1, IgG2, IgG3, and IgG4 (10). Among these, food-specific IgG4 (FS-IgG4) has been considered a potential clinical indicator for allergic symptoms (11).

The detection of FS-IgG4 appeared in the 1970s (12) and was soon applied in clinical work. However, the significance of IgG4 in allergic diseases is still controversial. It was not recommended for the diagnosis of food allergy in the most influential guidelines on food allergy, including those from the European Academy of Allergy and Clinical Immunology (EAACI) (13), the National Institute of Allergy and Infectious Diseases (NIAID) (14) and the American Academy of Allergy and Immunology Position Statement (AAAI) (15). These opposing opinions have clearly hindered the development of its application in clinical work until the studies on EOE changed the perception. Subsequent evidence has shown that FS-IgG4 is closely correlated with allergic diseases, including allergic rhinitis, asthma, atopic dermatitis, and chronic rhinosinusitis (16–19).

In the current study, we conducted a retrospective cross-sectional analysis utilizing data obtained from allergic patients aged 0-14 years. We assessed the positivity rates of total IgE, house dust mite (HDM) IgE, FS-IgE, and FS-IgG4 in various allergic systemic diseases. Furthermore, we identified and selected patients who underwent treatment involving FS-IgG4-guided diet elimination, with or without probiotics, for a duration exceeding 3 months and compared changes in clinical symptoms before and after treatment. Our investigation delves into pivotal questions, probing the relationships between FS-IgG4 and allergic diseases, and shedding light on the role of FS-IgG4 in allergic symptoms. The implementation of diet elimination guided by FS-IgG4 holds significant clinical relevance for controlling allergic symptoms in children. This study not only resolves existing uncertainties regarding FS-IgG4 but also provides novel clinical evidence for future research on its immunological mechanisms.

Methods

Study design and patients' selection

This retrospective observational study aimed to evaluate the clinical significance of serum total IgE, HDM IgE, FS-IgE, and FS-IgG4 antibodies in the treatment of allergic diseases in children. Electronic medical records (EMR) of all patients with allergy diseases treated at the Department of Allergy, the Second Affiliated Hospital of Zhejiang University School of Medicine from January 2018 to December 2020 were collected and evaluated. The inclusion criteria were children aged 0-14 years who fulfilled the ARIA guideline for allergic rhinitis and/or GINA guideline for asthma (20, 21), the International Consensus (ICON) guideline for conjunctivitis (22), the EAACI/GA2 LEN/EDF/WAO guideline for urticaria (23), the diagnostic criteria by Hannifin and Rajka for atopic dermatitis (24), and with the data of serum total IgE, HDM IgE, FS-IgE, and FS-IgG4 tested simultaneously. Patients with autoimmune diseases were excluded. Ultimately, 407 patients were recruited for the analysis. Among them, 67 patients underwent dietary elimination guided by FS-IgG4 and supplemented with/without probiotics. After more than 3 months of treatment, serum total IgE, HDM IgE, FS-IgE, and FS-IgG4 were retested, and clinical manifestations were reevaluated. The study flowchart is shown in Figure 1.

Study groups

According to the system affected, patients were categorized into three groups: the group with involvement of the respiratory system

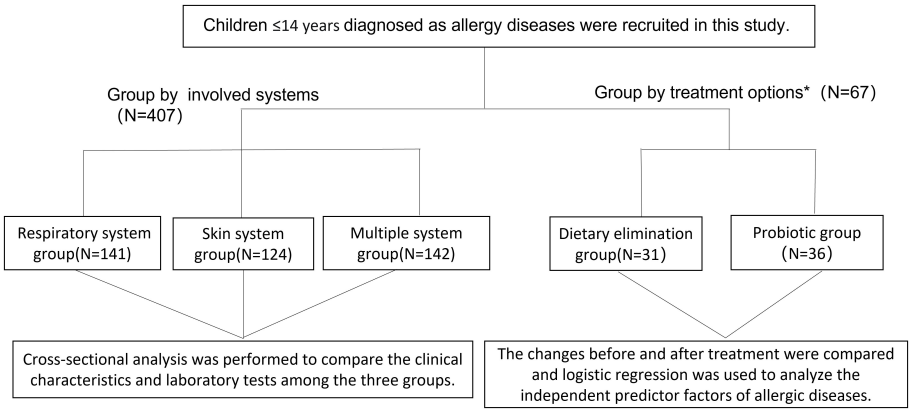

FIGURE 1
Flow chart of study participants. *Patients who were FS-IgG4 positive at initial diagnosis and had follow-up records for more than 3 months of treatment were selected.

(including rhinitis, asthma, and conjunctivitis), the group with involvement of the skin system (including urticaria and atopic dermatitis), and the group with multiple systems involvement (those with a combination of symptoms from different systems). Furthermore, patients with a follow-up history were divided into two groups based on their treatment regimen: those who underwent FS-IgG4-guided diet elimination for more than 3 months were assigned to the dietary elimination group, and those who underwent diet elimination combined with probiotic were assigned to the probiotic group.

Study variables and laboratory testing

Clinical characteristics and demographic profiles were obtained from the hospital's EMR system. Peripheral blood samples were taken from patients at the time of the initial presentation and during treatment according to the patients' condition. All laboratory tests were carried out in our hospital laboratory. Serum total IgE was measured by colloidal gold immunochromatography (Siemens Healthcare Diagnostics Products Limited, United Kingdom), HDM IgE and fifteen species of FS-IgE, including egg, milk, beef, crab, shrimp, cashews, mangoes, lamb, shellfish, lobster/scallops, cod, salmon, peanuts, beans, and pineapple, were detected by immunoblotting (Hangzhou Zheda Dixun Biological Gene Engineering Co., Ltd, China). All of these were expressed in international units per unit volume (IU/ml). Levels of total IgE value>100IU/ml, HDM IgE and FS-IgE value>0.35 IU/ml were considered positive. Ten species of FS-IgG4, including cod, egg, milk, beef, shrimp, soy, wheat, chicken, crab, and mushroom, were determined by enzyme-linked immunosorbent assay (Hangzhou Zheda Dixun Biological Gene Engineering Co., Ltd, China). The concentration of FS-IgG4 was divided into 4 grades: negative (−, <250 U/ml), weakly positive (+,250-500U/ml), positive (++,500-1000U/ml), strong positive (+++,>1000U/ml).

Diet elimination and probiotic supplement

Sixty-seven patients underwent diet elimination based on the result of FS-IgG4. Foods determined as ++ or +++ were completely forbidden to eat, and + foods were reduced in frequency of intake (eating the food at an interval of more than 4 days). Among them, 36 of the patients were supplemented with probiotics in addition to dietary elimination. The viable bacteria were composed of *Lactobacillus paracasei LP33, Lactobacillus fermentum GM090* and *Lactobacillus acidophilus GMNL-185* (GenMont Biotec Inc.). The above treatment time was required to last more than 3 months.

Outcome measure

Demographic data, serum level of total IgE, HDM IgE, FS-IgE, and FS-IgG4 from the three groups involving different systems were analyzed initially and at follow-up visits after varying periods of intervention (3-6 months, 6-9 months, 9-12 months, or over 12 months) with diet elimination, with/without probiotic supplementation. Laboratory-related indicators, such as total IgE and HDM IgE, were numerically compared. Due to the low positive frequency of FS-IgE, only the overall positive rate was used for statistical comparison. For FS-IgG4, the test data may exceed the kit reference range. The intervention of diet elimination or probiotic supplement caused distinct changes in different FS-IgG4; for example, one may decrease, but another may increase. Therefore, we designed a point system for statistical testing. We used 1 point to stand for one kind of elevated FS-IgG4, so patients' FS-IgG4 status was scored according to the number of foods with positive FS-IgG4. The total was then used to compare the difference between pre- and post-intervention. The same scoring method was applied to FS-IgE changes before and after treatment. Furthermore, the improvement of clinical symptoms was classified based on the subjective feelings of patients before and after treatment by comparing the numbers in the dietary elimination and probiotic group showing significant

improvement ('better 'or 'excellent'), and those with no significant change ('slightly worse ', 'no change', or 'slightly better'). Finally, we explored independent factors influencing the clinical manifestations of allergic diseases through regression analysis.

Statistical analysis

For all patients, continuous data following a normal distribution were expressed as mean ± standard deviation and compared using single-factor analysis of variance (ANOVA). Non-normally distributed data were expressed as median (interquartile range) and compared using the Kruskal-Wallis test. Categorical variables were expressed as frequencies (percentages) and compared using the Chi-square or Fisher's exact test. For patients before and after treatment, the paired t-test was used for normally distributed data, and the Wilcoxon signed-rank test was used for non-normally distributed data. The Student's t-test was employed to compare normally distributed data, while the Mann-Whitney U test was used for non-normally distributed data between the two treatment groups. Logistic regression analysis was conducted to determine whether the changes in total IgE, HDM IgE, FS-IgE, and FS-IgG4 before and after treatment were independent predictors for improvement of allergic diseases in children. Variables with an adjusted p value<0.1 in the univariate analysis were subsequently evaluated using a multivariate logistic regression model. A p-value <0.05 was the criterion for statistical significance in this analysis. Statistical analysis of all data was performed using SPSS 26.0 (IBM Corp.) and GraphPad Prism 8.0.1.

Results

Demographic characteristics

A total of 407 patients, with an average age of 7.2 ± 3.6 years (range 8 months to 14 years), were recruited and divided into three groups as following: 141 patients in the group with respiratory system involvement, 124 in the skin system group and 142 in the group with multiple systems involved. The baseline characteristics of the three groups are shown in Table 1. There were no significant differences in gender, age, family history of allergy, or birth history among the three groups (p> 0.05). Patients with multiple system allergies had the highest serum total IgE level ($p < 0.05$), and the lowest level of HDM IgE was observed in patients of the skin system ($p < 0.01$). The positive rate of FS-IgE was only about 30%, and there was no significant difference among the three groups. However, the positive rate of FS-IgG4 was more than 80 percent, and no significant difference among the three groups was found.

The difference in total/FS-IgE and FS-IgG4 positive rates among the three groups

In the three groups, we measured the proportion of patients who were positive for both total/FS-IgE and FS-IgG4, positive for total/FS-IgE but negative for FS-IgG4, negative for total/FS-IgE but positive for FS-IgG4, and negative for both. From the distribution of each group, the proportion of patients with both total IgE and FS-IgG4 positive was the highest, and the proportion of both negative

TABLE 1 Clinical and demographic characteristics of the study population.

Characteristics	Respiratory system group	Skin system group	Multiple system group	P
Patients, n	141	124	142	
Age (years), Mean ± SD	7.4 ± 3.5	6.7 ± 3.9	7.3 ± 3.3	0.093
Gender, n (%)				
Male	97 (68.8)	97 (68.8)	83 (58.5)	0.065
Female	44 (31.2)	55 (44.4)	55 (44.4)	
Mode of birth, n (%)				
Maternity leave	74 (52.5)	77 (62.1)	78 (54.9)	0.194
Cesarean	60 (42.6)	45 (36.3)	56 (39.4)	
Premature delivery	7 (5.0)	2 (1.6)	8 (5.6)	
Family history of allergy, n (%)				
Yes	54 (38.3)	48 (38.7)	59 (41.5)	0.833
NO	87 (61.7)	76 (61.3)	83 (58.5)	
Total IgE (IU/ml), Median (Q1-Q3)	185.0 (68.13-480.5)	126.0 (49.7-328.0) #	204.0 (78.8-549.0) #	0.033*
HDM IgE (IU/ml), Median (Q1-Q3)	2.75 (0.34-9.05)	0.34 (0.25-1.98)	2.2 (0.34-9.7)	0.003**
Positive rate of FS-IgE, n (%)	52 (36.9)	30 (24.2)	41 (28.9)	0.073
Positive rate of FS-IgG4, n (%)	121 (85.8)	100 (80.6)	119 (83.8)	0.631

Values are presented as mean ± SD or as absolute numbers (percentage). Non-normally distributed data are expressed as median (interquartile range). (*p<0.05). Pairwise comparisons (respiratory system group vs. skin system group; respiratory system group vs. multiple system group or skin system group vs. multiple system group, #p<0.05).

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TABLE 2 The positive rates of serum total IgE and FS-IgG4 in children with allergic diseases involving different systems.

Groups	Total IgE (+) FS-IgG4 (+) (%, n)	Total IgE (+) FS-IgG4 (–) (%, n)	Total IgE (-) FS-IgG4 (+) (%, n)	Total IgE (-) FS-IgG4 (-) (%, n)	X^2	P
Respiratory system group	58.2 (n=82)	9.9 (n=14)	27.7 (n=39)	4.3 (n=6)	13.36	0.039*
Skin system group$^{\#}$	51.6 (n=64)	9.7 (n=12)	29.8 (n=37)	8.9 (n=11)		
Multiple system group$^{\#}$	71.1 (n=101)	7.0 (n=10)	16.9 (n=24)	4.9 (n=7)		

Pairwise comparisons (respiratory system group vs. skin system group; respiratory system group vs. multiple system group or skin system group vs. multiple system group, $^{\#}$ $p<0.05$). (*$p<0.05$).

was the lowest. It is noteworthy that the proportion of patients with FS-IgG4 positive and total IgE negative was higher than that of patients with total IgE positive and FS-IgG4 negative. Meanwhile, we found that the distribution of proportions among the three groups was significantly different ($p<0.05$) (Table 2). Upon pairwise comparison between groups (according to the multiplicity test criteria), the proportion of patients with both positive results in the multiple system group was significantly higher than that in the skin system group ($p=0.012$). Different from total IgE, the positive rates of FS-IgE and FS-IgG4 were not significantly different among all groups (Table 3).

Comparison of FS-IgG4 positive rates among the three groups

The positive rate of each assayed FS-IgG4 of 10 kinds was analyzed and compared among the three groups. Detailed data are shown in Table 4. In all the groups, eggs had the highest positive rate, followed by milk, while mushrooms were not detected as positive. Meanwhile, we found that children with respiratory allergic diseases had the highest positive rate of milk, which was significantly different from that of the skin system ($p<0.01$). However, the positive rates of wheat and soybean in the multiple system group were the highest, which were significantly higher than that in the skin system group ($p<0.05$) and the respiratory system group ($p<0.05$) respectively.

Outcome before and after treatments

Of the 407 participants, 67 underwent at least one follow-up evaluation and repeated laboratory testing under diet elimination guided by FS-IgG4 with or without probiotic supplements. The follow-up interval of patients in both groups before and after treatment was more than 3 months, with the longest follow-up interval being two and a half years. There were no significant differences in total IgE, HDM IgE, FS-IgE, and FS-IgG4 between the dietary elimination group and the probiotic group before treatment. However, after treatment, there was a significant difference in FS-IgG4 between the two groups, and the decrease was more pronounced in the dietary elimination group. Total IgE, FS-IgE, and HDM IgE remained unchanged (Figure 2). In addition, no significant differences before and after treatment were noted with regard to the changes in the total IgE, FS-IgE, and HDM IgE between the dietary elimination group and probiotic group. Yet, we found a significant decrease in FS-IgG4 before and after treatment in both groups. Comparatively, a more significant decrease was observed in the dietary elimination group (Figure 3). Moreover, the patients were assigned to four groups according to the follow-up interval, which was 3-6 months, 6-9 months, 9-12 months, and > 12 months. At different follow-up intervals, FS-IgG4 decreased most significantly in patients who were followed up for 9-12 months, followed by 3-6 months and 6-9 months, while FS-IgG4 did not decrease significantly in patients longer than 12 months (Figures 4A, B, D, E). Similarly, the clinical symptoms of patients in both groups were improved dramatically. Although there were no significant differences between the two groups, the percentage of patients with a significant improvement was 67.7% (n=21) in the dietary elimination group and 77.8% (n=28) in the probiotic group, with the remainder having no significant responses (Figure 4C).

The results of binary logistic regression analysis

In order to find the predictable index of the improvement of clinical symptoms, we used a multivariate logistic regression model to test different variables including age, gender, birth history, family history, use of probiotics supplement or not, and the changes of

TABLE 3 The positive rates of serum FS-IgE and FS-IgG4 in children with allergic diseases involving different systems.

Groups	FS-IgE (+) FS-IgG4 (+) (%, n)	FS-IgE (+) FS-IgG4 (-) (%, n)	FS-IgE (-) FS-IgG4 (+) (%, n)	FS-IgE (-) FS-IgG4 (-) (%, n)	X^2	P
Respiratory system group	34.0 (n=47)	2.8 (n=5)	51.8 (n=73)	11.3 (n=16)	6.922	0.32
Skin system group	19.4 (n=24)	4.8 (n=6)	61.3 (n=76)	14.5 (n=18)		
Multiple system group	26.8 (n=37)	2.1 (n=5)	57.0 (n=80)	14.1 (n=20)		

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TABLE 4 The positive rates of the FS-IgG4 in three groups. (*p<0.05).

Groups	Respiratory system group	Skin system group	Multiple system group	P
Egg (%, n)	81.6 (n=115)	75.8 (n=94)	79.6 (n=113)	0.51
Milk (%, n)	70.9 (n=100) #	54.8 (n=100) #	64.1 (n=91)	0.025*
Wheat (%, n)	19.1 (n=27)	12.1 (n=15) #	26.1 (n=37) #	0.0163*
Gadus (%, n)	18.4 (n=26)	14.5 (n=18)	25.4 (n=36)	0.0776
Soybean (%, n)	7.8 (n=11) #	13.7 (n=17)	19.0 (n=27) #	0.0224*
Shrimp (%, n)	3.2 (n=4)	3.2 (n=4)	6.3 (n=9)	0.2775
Crab (%, n)	2.1 (n=3)	3.2 (n=4)	6.3 (n=9)	0.2086
Beef (%, n)	2.8 (n=4)	2.4 (n=3)	4.9 (n=7)	0.4751
Chicken (%, n)	3.5 (n=5)	2.4 (n=3)	6.3 (n=9)	0.2532
Mushroom (%, n)	0 (n=0)	0 (n=0)	0 (n=0)	–

Pairwise comparisons (respiratory system group vs. skin system group; respiratory system group vs. multiple system group or skin system group vs. multiple system group, # p<0.05).

total IgE, HDM IgE, FS-IgE, and FS-IgG4. The results showed that the change in FS-IgG4 was an independent predictor for significant improvement of clinical symptoms in children with allergies (adjusted OR: 1.412,95% CI 1.017–1.96, p=0.039; Figure 4F).

Discussion

The current study is the first clinical investigation to confirm the clinical correlation of FS-IgG4 antibodies with allergic diseases through the intervention of FS-IgG4-guided diet elimination. We observed elevated serum FS-IgG4 levels in allergies of different systems and analyzed the expression of different FS-IgG4 cations for 10 common foods. Moreover, we further followed up on these patients for at least 3 months with the intervention and found that FS-IgG4-guided diet elimination significantly improved the allergic symptoms.FS-IgG4 also emerged as an independent predictor for clinical improvement.

In our study, we observed a significantly higher positive rate of FS-IgG4 compared to FS-IgE in allergic children. Measurement of total IgE, HDM IgE, FS-IgE, and FS-IgG4 revealed that most patients exhibited positive total IgE and FS-IgG4, with a very low positive rate of FS-IgE. This finding aligns with previous studies (14), in spite of the rapid increase in food allergy in children. Our results suggest a potentially more crucial role for FS-IgG4 in allergic rhinitis/asthma or urticaria and AD among Chinese children, indicating that the immediate response mediated by FS-IgE might not be the primary cause of these chronic allergies. Furthermore, the dual positivity of IgE and FS-IgG4 is significantly higher in the group of multiple systems, implying a correlation between

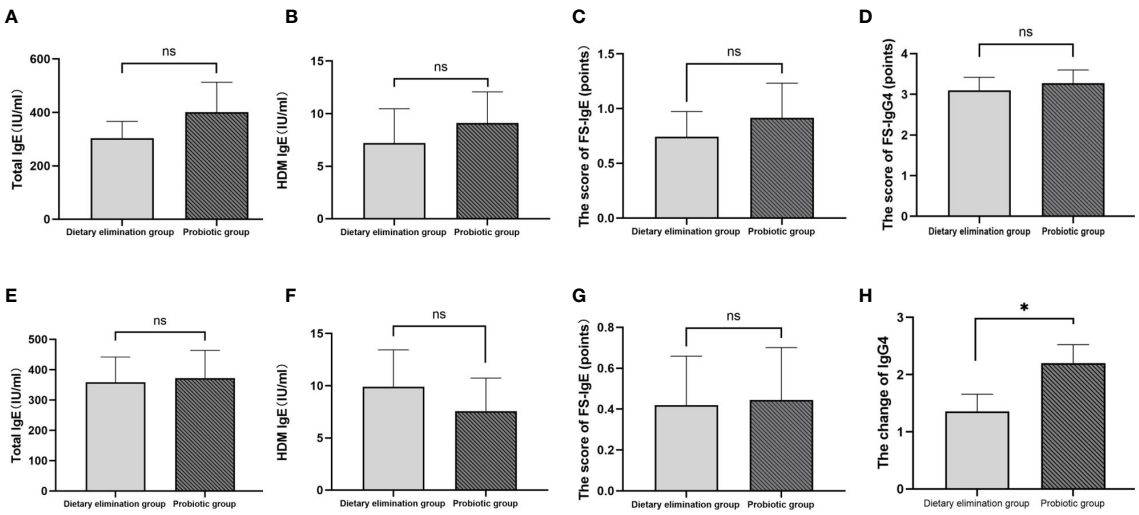

FIGURE 2
Comparison of total IgE, HDM IgE, FS-IgE, and FS-IgG4 between the dietary elimination group and the probiotic group before **(A-D)** and after treatment **(E-H)** (*p<0.05). ns, no significant.

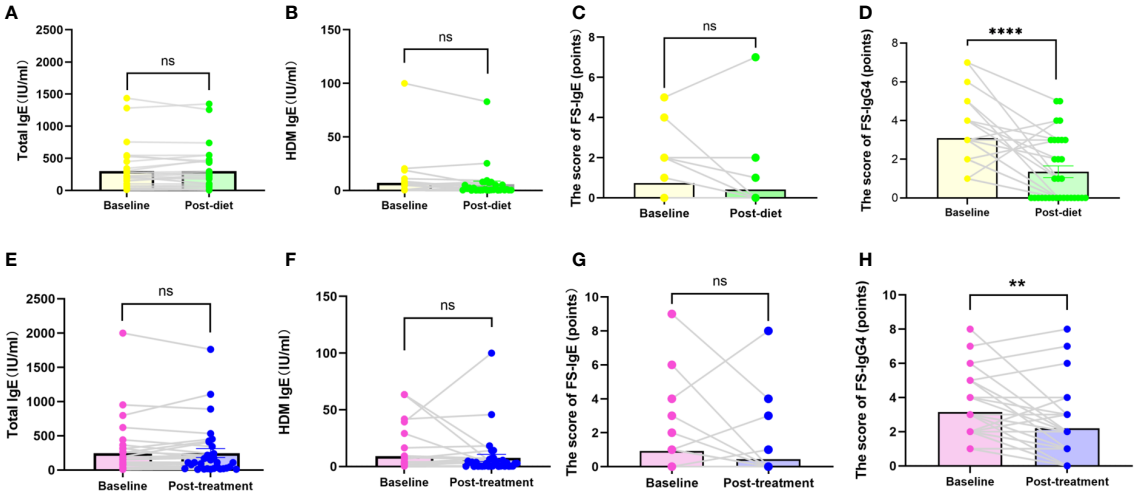

FIGURE 3
Comparison of total IgE, FS-IgE, HDM IgE, and FS-IgG4 in baseline and after treatment in the dietary elimination group **(A-D)** and the probiotic group **(E-H)**. **P<0.01; ****P<0.0001; ns, no significant.

the number of systems affected and the extent of immune reactions. Notably, the positive ratio of FS-IgG4(+) total IgE (–) is higher than FS-IgG4 (–) total IgE (+). Regardless of the system involved, more than 80% of allergic children exhibit a positive rate of FS-IgG4, while the positive rates of total IgE and FS-IgE are comparatively lower. Previous research has often explained the rapid development of FS-IgG4 in early-stage allergic children exposed to food as a normal physiological response (25, 26)., leading to its clinical significance being overlooked. However, in healthy children, the increase in serum IgG4 is very gradual, reaching adult levels only around puberty (27). Therefore, any elevation beyond normal adult levels can be considered potentially impactful.

Further analysis of the positive rates of FS-IgG4 for different foods revealed that FS-IgG4 levels were highest for egg and milk. This is likely attributed to greater exposure to these two types of foods during childhood. Milk is also the leading cause of food allergies (14). This meets the explanation of a previous study by *Millers etc.* Their research on EOE demonstrated a close association with IgG4 antibody levels in milk and wheat (28). Given that many children with allergic diseases continue to consume large amounts of eggs, milk, wheat products, and soy milk for growth needs, it is not surprising that the corresponding rise in FS-IgG4 antibodies was significant. Researches confirm that continuous exposure to allergens induces eosinophilic inflammation in the esophagus with

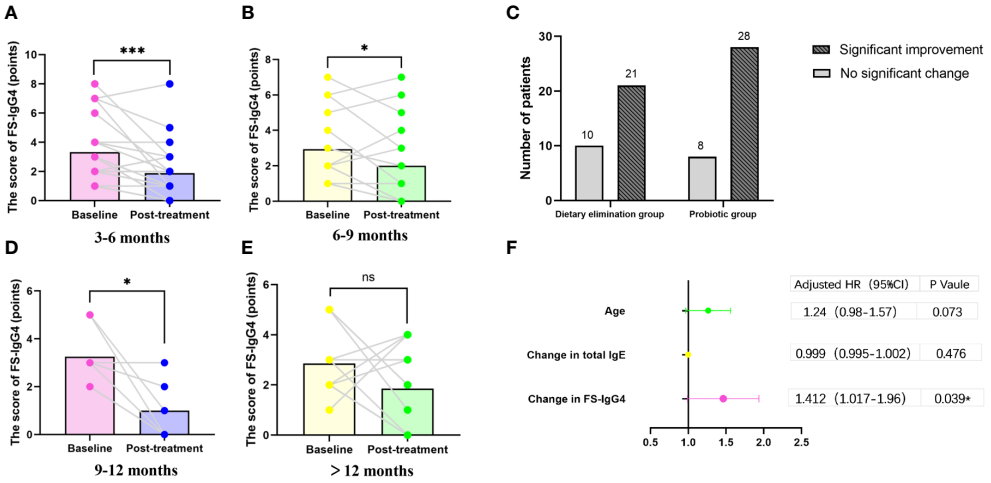

FIGURE 4
For all the treated patients (N=67), FS-IgG4 changes according to different follow-up intervals **(A, B, D, E)** (*p<0.05). The proportion of patients with improvement in clinical symptoms in both groups before and after treatment **(C)** (*p<0.05); Forest plot of outcomes in study population (N=67). Outcomes are presented as odds ratios with 95% confidence intervals. OR<1 favors no significant remission, OR>1 favors alleviating. Univariate analysis demonstrated children with allergies whose FS-IgG4 decreased more had a higher rate of clinical remission **(F)**. (*p<0.05); ***P<0.001; ns, no significant.

locally significant IgG4 deposition in patients with EOE (29) and patients receiving oral immunotolerance therapy (30). Nevertheless, dietary elimination as a treatment for EOE has been shown to have positive effects in both adults and children (31).

The intervention of diet elimination guided by FS-IgG4 in our study also demonstrated a significant improvement in symptoms correlation with serum indices, in accordance with previous research on EOE. Serum FS-IgG4 decreased significantly after diet elimination, accompanied by a substantial improvement in symptoms. Regression analysis indicated that the reduction in FS-IgG4 was an independent predictor affecting the degree of clinical symptom remission in allergic children. Neither total IgE nor FS-IgE changed in spite of the dramatic improvement in symptoms, further confirming that FS-IgG4 played a more critical role in these allergic reactions, whether in the skin or respiratory systems. These observations were in keeping with very recent studies that eliminated allergic foods based on FS-IgG antibodies in patients with irritable bowel syndrome, Crohn's disease, and migraine, all of which demonstrated relief of related symptoms (32–36).

Compared to another intervention of probiotic supplements as add-on strategies, the changes of FS-IgG4 are more pronounced in children with diet elimination. However, the relief ratio of clinical symptoms of allergies is more efficient in the probiotic group, with 77.8% compared to 67.7% in the diet elimination group. This may result from the multiple impacts of probiotics in relieving clinical symptoms besides the modulation of antibody production. The relief of clinical symptoms of probiotics led to less strict diet elimination, resulting in a smaller decrease in FS-IgG4 compared to children undergoing only diet elimination. The changes in serum FS-IgG4 within a year of follow-up duration provide interesting evidence to verify this hypothesis. We observed a lasting decrease in FS-IgG4 with the duration of diet elimination, followed by an increase at the 1-year time point. We speculate that children who experience sufficient relief under short-term diet elimination often discontinue therapy and reintroduce the food due to concerns about nutritional deficiencies.

In this study, we have observed a close relationship between FS-IgG4 and allergic symptoms in children. After dietary elimination guided by FS-IgG4, clinical symptoms improved significantly, indicating that the occurrence of allergic symptoms in children is induced by both IgG and IgE and may even occur independently of IgE. In classical allergic sensitization, IgE-producing plasma cells are generated, and initial symptoms may stem from IgG-producing B cells reacting to allergens. The IgG-to-IgE class switching process primes mast cells. However, numerous allergic reactions can occur independently of allergen-specific IgE, even in the absence of total IgE. IgG, contributing to Th2 polarization, enhances allergic responses. Allergy processes go beyond specific IgE, with IgG influencing atopy, clinical symptoms, and the resolution of allergies. Importantly, the pattern of IgG-producing plasma cells in atopic children signifies crucial events leading to enduring mast cell sensitization in allergies (37). In previous studies, it has been detected that IgE and IgG antibodies share similar portions, indicating common antigen secretion triggers that can bind to similar antigenic epitopes, particularly in patients with peanut or milk allergies (38, 39). Similar results have also been validated in patients with wheat and soy allergies (40, 41). In the comorbidity analysis of epitope-specific antibodies in children with egg allergies, it was observed that nearly all elevated levels of epitope-specific IgG4 antibodies were associated with rhinitis. In a model adjusted for asthma and age, one IgG4 epitope exhibited a significant correlation (42). This evidence suggests the presence of IgE conversion mechanisms, including the production of IgG, in patients with food allergies. In both mice and humans, evidence suggests that allergies or inflammatory responses can be mediated by IgG (43, 44), indicating that the onset of allergies may be independent of IgE and instead triggered by IgG or alternative pathways.

In conclusion, serum FS-IgG4, but not FS-IgE, is found to be correlated with allergic diseases more significantly than previously recognized. Eggs and milk emerge as the most common allergens influencing the development of allergic symptoms. Diet elimination guided by FS-IgG4 proves to be an effective method for managing allergic diseases in children. Our study thus contributes solid data to the understanding of the role of FS-IgG4 in allergic diseases. Our findings hold the potential to advance the comprehension of the clinical significance of FS-IgG4 in allergic diseases and provide valuable insights for the diagnosis and treatment of pediatric allergic conditions.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the second affiliated hospital of Zhejiang university school of medicine (No. 2022-0380). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

BY: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. HY: Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. WY: Conceptualization, Investigation, Methodology, Project administration, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Unveiling the dynamics of gut microbial interactions: a review of dietary impact and precision nutrition in gastrointestinal health

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The human microbiome, a dynamic ecosystem within the gastrointestinal tract, plays a pivotal role in shaping overall health. This review delves into six interconnected sections, unraveling the intricate relationship between diet, gut microbiota, and their profound impact on human health. The dance of nutrients in the gut orchestrates a complex symphony, influencing digestive processes and susceptibility to gastrointestinal disorders. Emphasizing the bidirectional communication between the gut and the brain, the Brain-Gut Axis section highlights the crucial role of dietary choices in physical, mental, and emotional well-being. Autoimmune diseases, particularly those manifesting in the gastrointestinal tract, reveal the delicate balance disrupted by gut microbiome imbalances. Strategies for reconciling gut microbes through diets, precision nutrition, and clinical indications showcase promising avenues for managing gastrointestinal distress and revolutionizing healthcare. From the Low-FODMAP diet to neuro-gut interventions, these strategies provide a holistic understanding of the gut's dynamic world. Precision nutrition, as a groundbreaking discipline, holds transformative potential by tailoring dietary recommendations to individual gut microbiota compositions, reshaping the landscape of gastrointestinal health. Recent advancements in clinical indications, including exact probiotics, fecal microbiota transplantation, and neuro-gut interventions, signify a new era where the gut microbiome actively participates in therapeutic strategies. As the microbiome takes center stage in healthcare, a paradigm shift toward personalized and effective treatments for gastrointestinal disorders emerges, reflecting the symbiotic relationship between the human body and its microbial companions.

KEYWORDS

microbiome, gastrointestinal health, precision nutrition, gut-brain axis, autoimmune diseases

Introduction

The human body often likened to a complex ecosystem, vividly exemplifies this analogy in the form of the gut microbiome (1). Within the intricate landscape of the human digestive system, a bustling community of microorganisms, encompassing bacteria, viruses, fungi, and more, collectively orchestrates a symphony that profoundly influences human health and

well-being (2, 3). In the context of article, "symphony" likely refers to the intricate and coordinated interactions within the gastrointestinal tract involving diet, gut microbiota, and their impact on health. The gut microbiome, a dynamic and diverse population, engages in a multifaceted relationship with its human host, contributing to various physiological processes and serving as a pivotal player in maintaining equilibrium (4). This microbial ecosystem within the digestive tract is not a static entity but rather a living, evolving ecology shaped by an interplay of factors such as genetics, diet, environment, lifestyle, and even the mode of delivery at birth (5, 6). The makeup of this intricate microbiome is composed of thousands upon thousands of microbial species delicately existing in a precarious equilibrium. The gut, acting as the primary residence for this diverse microbial community, houses an array of microorganisms, predominantly bacteria, alongside viruses, archaea, and eukaryotic species (1). Far from a passive bystander, the gut microbiota actively participates in key physiological processes that impact human health. Its role extends to the intricate breakdown of complex carbohydrates, proteins, and fats that might challenge the body's enzymes (7). The trillions of bacteria populating the digestive tract play a vital role in breaking down these molecules into absorbable forms, facilitating nutrient absorption (8). Moreover, the gut microbiota exerts influence over metabolic processes, affecting energy storage, nutrient processing, and appetite regulation. A symbiotic relationship is evident in the microbiota's contribution to immune system modulation. By conditioning the immune system to respond effectively to harmful pathogens while curbing unnecessary inflammation, the gut microbiota acts as a crucial ally in maintaining immune balance (9). Additionally, certain microbial inhabitants are involved in the synthesis of essential vitamins B and K, as well as short-chain fatty acids (SCFAs) renowned for their anti-inflammatory properties (10, 11). Preserving the delicate equilibrium of the gut microbiome, termed dysbiosis-(disruption in the gut microbiota composition when disrupted), is paramount for overall health. Dysbiosis has been linked to various diseases and conditions, including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), obesity, and certain neurological disorders (12). Therefore, maintaining a diverse and stable microbial community is integral to promoting a healthy gut microbiome. Dietary choices emerge as a powerful tool in shaping the gut microbiome. Consuming meals rich in dietary fiber fosters an environment conducive to the thriving of beneficial bacteria. Prebiotic foods, encompassing fibers that serve as sustenance for beneficial bacteria, contribute to microbial diversity and further support gut health. In essence, the gut microbiome stands as a complex ecosystem with far-reaching effects on human health (13). From its pivotal role in digestion to its contribution to immune function, understanding the profound symbiotic link between humans and their microbial inhabitants underscores the significance of the gut microbiome. Elevating awareness of its importance and making informed dietary choices to promote diversity within this microbial community hold the potential to enhance health outcomes and deepen our comprehension of this intricate relationship. This burgeoning field of microbiome research is poised to transform our approach to human health, paving the way for innovative therapeutic interventions and personalized treatments targeting the gut microbiome. As we delve deeper into the intricacies of microbiome dynamics in human diseases, the potential for groundbreaking discoveries and therapeutic breakthroughs becomes increasingly apparent.

Navigating the nutrient landscape: impact on gut microbiota

The intricate relationship between diet and gut microbiota has emerged as a pivotal determinant in the multifaceted landscape of human health (Figure 1). Both our digestive processes and susceptibility to gastrointestinal disorders are profoundly influenced by the dynamic interplay between dietary components and the microbial inhabitants of the gastrointestinal tract (14).

Food and bacteria as a complicated Tango

The composition of our gut microbiota is directly influenced by the nutrients we consume. The gut microbiota can respond in various ways to different components of the diet, including carbohydrates, proteins, lipids, fibers, and specific bioactive chemicals. Complex carbohydrates, such as dietary fiber, serve as a crucial source of fuel for certain beneficial bacteria. The fermentation of fiber by these bacteria produces short-chain fatty acids (SCFAs)—which are organic acids produced by gut bacteria during the fermentation of dietary fiber (Table 1), which possess anti-inflammatory properties and contribute to gut health (15, 16). Proteins from the diet can impact gut microbial diversity, with high-protein diets potentially encouraging the growth of bacteria that utilize amino acids, leading to the generation of harmful compounds. The types and quantities of fats in one's diet significantly affect the composition of the gut microbiome, potentially influencing bacterial imbalances linked to obesity and metabolic diseases (17). Foods rich in fiber and prebiotic ingredients sustain beneficial bacteria and foster a healthy microbiome, playing a crucial role in preventing and treating digestive disorders (18). Polyphenols and phytochemicals, plant-based molecules with antioxidant and anti-inflammatory characteristics, can positively influence the gut flora (19).

Consequences for digestive disorders

The intricate dance between nutrition and gut flora has far-reaching effects on gastrointestinal disorders. Changes in gut microbial composition have been associated with conditions such as irritable bowel syndrome (IBS), Crohn's disease, ulcerative colitis, and gastroesophageal reflux disease (GERD) (20, 21). Dietary habits can either exacerbate or alleviate symptoms, emphasizing the potential for controlling and preventing gastrointestinal diseases through personalized dietary approaches (22).

Exposing the role of dietary fiber in feeding good bacteria

The significance of dietary fiber in nurturing a robust gut flora and maintaining overall gut health is often underestimated despite its widely recognized positive effects on digestion. This humble substance is more than roughage; it is a vital component of the digestive system's orchestra, playing a crucial role in: Fiber serves as a powerful prebiotic, feeding beneficial bacteria in the digestive tract, producing SCFAs that reduce inflammation, fortify the gut barrier, and enhance overall gut health (23). Eating a variety of fiber-rich foods promotes a healthy

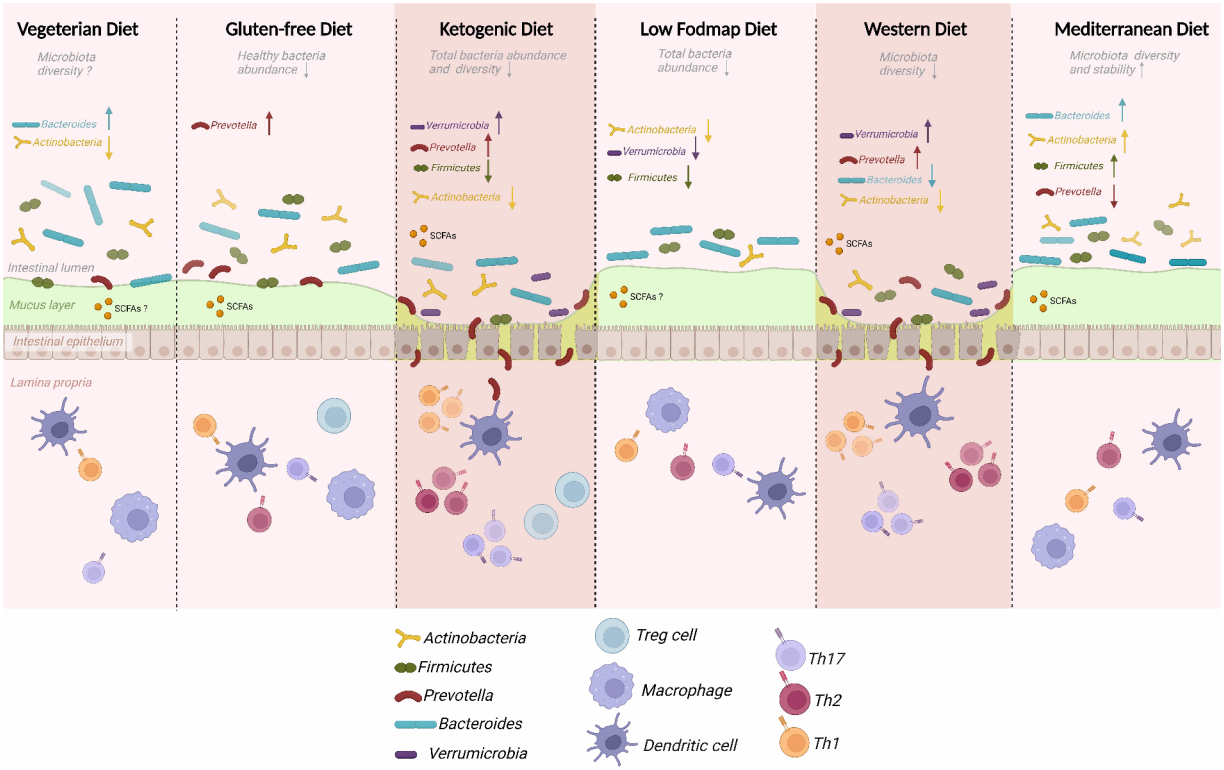

FIGURE 1
Dietary impact on gut microbiota composition.

TABLE 1 Metabolites from food and their associations with gut microbial communities: additional review points.

Metabolite	Food Source	Microbial association
Short-chain fatty acids (SCFAs)	Fiber-rich foods such as fruits, vegetables, and whole grains	Produced by gut microbiota through fermentation of dietary fiber
Polyphenols	Fruits (e.g., berries), vegetables, tea, red wine	Metabolized by gut bacteria into bioactive compounds with antioxidant and anti-inflammatory properties
Tryptophan	Protein-rich foods such as turkey, chicken, eggs, nuts, and seeds	Metabolized by gut bacteria into serotonin and other neurotransmitters, influencing mood and cognitive function
Sulfur-containing compounds (e.g., hydrogen sulfide)	Cruciferous vegetables (e.g., broccoli, cabbage), garlic, onions	Production by sulfate-reducing bacteria in the gut, implicated in gastrointestinal health and disease

balance of microorganisms in the gut (13). Insoluble fiber from whole grains and vegetables aids in relieving constipation by increasing stool volume, while soluble fiber from oats and lentils helps maintain regular bowel movements.

The effect of fiber on digestive disorders

Increased consumption of soluble fiber has been reported to improve symptoms in some individuals with IBS (24). Dietary fiber may positively impact inflammatory bowel disease (IBD) by altering the gut microbiome, although the extent varies based on the condition and circumstances. A high-fiber diet is associated with a lower risk of diverticular disease and related issues like diverticulitis. Diets rich in fiber are linked to a decreased risk of colorectal cancer, attributed to regular bowel movements and increased SCFA production (25). Dietary fiber plays a pivotal role in maintaining digestive tract health, significantly influencing the gut microbiome's composition and function. By incorporating a diverse range of high-fiber foods into our diets, including whole grains, fruits, vegetables, and legumes, we not only improve our health but also provide nourishment for the microbes within us (26). The intricate and mutually beneficial interaction between our food choices and the remarkable biosphere within us is best exemplified by the fiber-microbiome partnership (27).

Studying the role of probiotic-rich foods and prebiotic fibers

The nutritional conductors of gut health, probiotics and prebiotics, orchestrate a symphony of interactions among the microflora in the digestive tract. The gut microbiome, a dynamic ecosystem, is

influenced by various dietary components performing unique yet interconnected roles. Probiotics, beneficial bacteria and live microorganisms, and prebiotics, indigestible dietary fibers, offer distinct benefits: Probiotics restore diversity and balance to the gut microbiota, introducing helpful bacterial strains. Probiotics outcompete pathogenic microbes, reducing the risk of infection and gastrointestinal distress. Some probiotics interact with the immune system beneficially, leading to more even and potentially less inflammatory immune responses. The potential modulation of nutritional and energy metabolism by probiotics (28, 29).

Feeding the philharmonic with prebiotics

Prebiotics, indigestible dietary fibers providing food for beneficial bacteria, contribute to the synergy with probiotics: Prebiotics selectively target and increase the population of specific beneficial bacteria already present in the gut (30). The fermentation of prebiotics produces SCFAs, benefiting gut health by reducing inflammation, fortifying the gut barrier, and supplying energy for colonic cells. Certain prebiotic fibers help with gut motility by promoting regular bowel movements and relieving constipation. Combining prebiotics and probiotics produces a synergistic impact, enhancing health advantages by simultaneously nourishing beneficial microorganisms (31). The gut microbiota is most at peace and resilient when probiotics and prebiotics work together: Combining the benefits of probiotic-rich meals with prebiotic fibers fosters a more diversified and stable microbiome. Improved intestinal permeability defenses are possible, thanks to prebiotic fermentation contributing to SCFA production. Maintaining stability in the gut microbiota, despite dietary or environmental changes, is facilitated by the synergistic effects of probiotics and prebiotics, protecting against dysbiosis (32). The nutritional symphony benefiting the entire gut flora is produced when probiotics and prebiotics work in harmony. These factors, akin to conductors, lead the microbial orchestra to greater unity, variety, and resistance. Improving gut health by incorporating more probiotic-rich foods and prebiotic fibers highlights the interconnectivity of our food choices and the thriving world of microorganisms within us.

The unsung hero in gut health

Traditional diets have given way to Western diets, defined by the prevalence of processed and convenience foods. This dietary transformation has significantly impacted the food landscape, prompting increased interest in vegetarian and vegan diets with a focus on unprocessed, natural foods (33). Ongoing research delves into the consequences of these dietary choices on gastrointestinal health and function. The Western diet, characterized by its consumption of processed and sugary foods, initiates a cascade of consequences affecting gut health (34). Notably, a decrease in microbial diversity is observed, potentially contributing to abnormalities in the gut microbiota and an increased susceptibility to gastrointestinal diseases (35). Consuming processed foods high in sugar and unhealthy fats may induce a dysbiotic and inflammatory state in the body, with conditions like IBD and IBS linked to inflammation caused by dysbiosis (36). The gut barrier can be compromised due to a diet rich in sugar and fat, potentially leading to toxin absorption and immune system activation. Conditions associated with altered gut microbiota composition, such as obesity and metabolic syndrome, are exacerbated by Western dietary patterns. In contrast, diets rich in plant-based whole foods, including fruits, vegetables, legumes, and grains, demonstrate several positive effects on gut health (37). Plant-based diets are high in fiber and support a diverse and beneficial microbiome by providing sustenance to various types of beneficial gut bacteria. Whole plant diets exhibit anti-inflammatory effects, potentially alleviating gastrointestinal inflammation associated with conditions like inflammatory bowel disease. The fiber in plant-based diets contributes to a stronger intestinal barrier, reducing the absorption of toxic chemicals into the bloodstream. Weight management and metabolic health are positively influenced by plant-based diets, potentially reducing the risk of obesity-related gastrointestinal diseases. While the disparities between Western and plant-based diets in their impact on gut health are evident, moderation remains crucial (38). A nuanced, plant-based diet that incorporates minimally processed foods can be more effective than a strictly binary approach. Our digestive tract's state and overall health are intricately linked to the foods we consume. The conflict between Western diets, high in processed foods, and plant-based diets, rich in whole, natural foods, underscores the pivotal role of diet in shaping our gut microbiota and overall health. Plant-based diets, coupled with mindful consumption of processed foods, set the stage for a robust gut microbiota, reinforced gut barrier, and a harmonious connection between our dietary choices and the complex ecosystem within us (39).

Gut microbiome's impact on gastrointestinal health

The intricate balance of our intestines is profoundly affected by the gut microbiome, a teeming ecology of microorganisms that inhabits our digestive tract. Microbial changes, disrupting the equilibrium of this microbial population, have been linked to several gastrointestinal problems. The IBS, IBD, and gastroesophageal reflux disease (GERD) are all illnesses that may be exacerbated by these changes: The recent study recruited 100 participants with diverse dietary habits and gut microbiome profiles. Participants were randomly assigned to either a personalized dietary intervention group or a control group following a standard dietary recommendation. The personalized intervention group received individualized dietary plans based on their gut microbiome composition, determined through comprehensive metagenomic analysis. The dietary plans were tailored to optimize the growth of beneficial microbial species and reduce the abundance of potentially harmful microbes. After a 12-week intervention period, fecal samples were collected for microbiome analysis, and participants underwent clinical assessments to evaluate changes in gut health markers. The results demonstrated significant improvements in gut microbiome diversity, composition, and metabolic function in the personalized nutrition group compared to the control group. Moreover, participants in the personalized intervention group reported reduced gastrointestinal symptoms and improved overall well-being. These findings underscore the potential of precision nutrition approaches in promoting gut microbiome

health and individualized dietary recommendations for optimal health outcomes (40).

Microbial imbalances

Catalysts for gastrointestinal disorders. Disturbances in the gut microbiota have been linked to IBS, a condition characterized by stomach pain, bloating, and altered bowel habits (41). IBD is characterized by persistent inflammation of the gastrointestinal system and includes Crohn's disease and ulcerative colitis (41). The microbial imbalance and decreased diversity known as dysbiosis are hallmarks of inflammatory bowel disease. The immune system's reaction to a dysbiotic pattern could worsen inflammation and hasten the development of disease. Acid reflux and heartburn are symptoms of GERD, which can be affected by changes in the microbiome. The synthesis of metabolites that have an impact on oesophagal health may be affected by bacterial imbalances in the gut (38). In addition, the lower oesophagal sphincter's function can be affected by these changes, heightening reflux symptoms.

The link between microbial changes and GI problems is mediated in several ways, symptoms and progression of gastrointestinal diseases can be influenced by inflammation and immunological responses, both of which can be triggered by dysbiosis. Changes in gut microbiota composition can weaken the intestinal barrier, enabling potentially dangerous chemicals to enter the body and set off an immunological response. Changes in microbes can affect the production of metabolites such as SCFAs, which affect inflammation and gastrointestinal health. The creation of neurotransmitters may have consequences for disorders like irritable bowel syndrome, and gut microorganisms play a role in this process (42). Managing microbial changes for gastrointestinal well-being, probiotics and prebiotics are used to increase the growth of beneficial bacteria, restore microbial balance, and reduce symptoms. Low-FODMAP diets for IBS is one example of a dietary intervention that has shown potential in the management of IBS symptoms by targeting certain microbial imbalances (43). Tailoring therapy to specific microbial imbalances may be possible with personalized interventions based on an individual's gut microbiome profile. The function of the gut microbiome in gastrointestinal illnesses is becoming clearer as our understanding of this complex ecosystem grows. Conditions like bowel disease, and gastroesophageal reflux disease have been linked to changes in the microbiome. The substantial connection between our gut microbiota and gastrointestinal well-being is being uncovered by academics and healthcare practitioners as they gain a better knowledge of these dynamics.

Decoding the brain-gut axis for holistic health

The Brain-Gut Axis researches the intricate connection between the gut and the brain, often referred to as the "second brain" (44). This relationship unveils how our food choices impact both physical and mental health. Nutrition emerges as a pivotal player in shaping gut flora, influencing conditions like depression and anxiety. The microbiome in the intestines communicates with the central nervous system, producing metabolites, neurotransmitters, and immunological chemicals. Notably, certain stomach bacteria generate vital neurotransmitters such as serotonin and dopamine (45). A healthy gut flora, nurtured by a diet rich in fiber, prebiotics, and probiotics, contributes to the production of mood-regulating neurotransmitters (Figure 2) (46). Conversely, diets high in processed foods and sugars can induce inflammation, affecting both the gut and the brain and potentially leading to mood disorders (38, 47). The intricate link between the gut and the brain underscores the profound impact of dietary choices on physical, mental, and emotional well-being, emphasizing the importance of embracing nutrient-dense diets and supporting a diverse gut flora for overall health (46).

Gut microbiome's role in autoimmune diseases

Autoimmune diseases often manifest in the gastrointestinal tract, with emerging evidence suggesting the crucial role of gut microbiome imbalances in their development (48). Celiac disease, characterized by gluten-triggered immune fibers reactions, showcases the interplay between genetics, the gut microbiome, and disease onset (49). Dysbiosis, or imbalances in the gut microbiome, can trigger immune dysregulation, creating a pro-inflammatory environment that intensifies the immune system's response to gluten. The consequential changes in microbial metabolite production and damage to the intestinal barrier can lead to conditions like a "leaky gut," where microbial components pass through, triggering immune responses against the host's tissues (50, 51). Altered microbial composition, reduced diversity, and abnormalities in bacterial groups are common in the gut microbiota of individuals with celiac disease (49). Recognizing the potential therapeutic role of gut microbiota modulation, interventions like probiotic supplements and dietary changes are explored. Tailoring treatments to individual microbial imbalances offers a promising avenue for managing autoimmune diseases, paving the way for innovative research and potential breakthroughs in understanding and treating these conditions.

Strategies for reconciling gut microbes through diet

Strategies for reconciling gut microbes through diet play a pivotal role in maintaining digestive tract health. The Low-FODMAP diet, proven effective in managing symptoms of conditions like IBS, targets fermentable carbohydrates to alleviate gastrointestinal issues. Introducing probiotics, beneficial microorganisms, to the diet modulates gut microbiota composition, enhances microbial diversity, and influences immune responses and gut barrier function (26, 40). Prebiotics, found in foods like garlic and bananas, provide indigestible fibers that stimulate the growth of beneficial microorganisms, creating anti-inflammatory short-chain fatty acids (52). The Mediterranean diet, rich in fiber and polyphenol-rich foods, showcases potential benefits against gastrointestinal disorders by enhancing gut flora diversity and balance (53). These dietary interventions offer pathways to microbial reconciliation and improved gut health, underlining the significant role of food in shaping the complex ecosystem of the gut

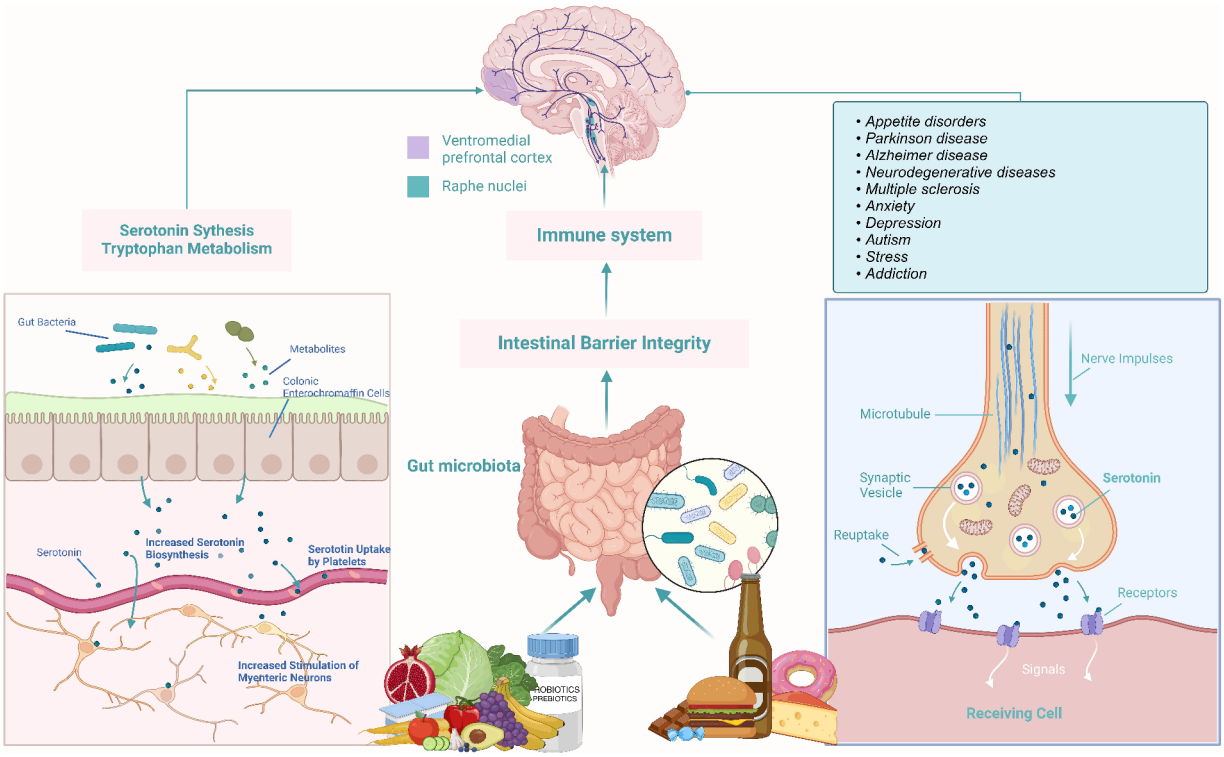

FIGURE 2
Brain-gut axis: impact of diet on mental health.

microbiota. As research progresses, these approaches may revolutionize the treatment of gastrointestinal distress, providing personalized and effective strategies for individuals based on their unique microbial composition (54).

Tailoring diets to microbial masterpieces

Precision nutrition is a groundbreaking scientific discipline reshaping our approach to health and wellness (Figure 3). By tailoring dietary recommendations to an individual's unique gut microbiota composition, this emerging field holds the potential to revolutionize the management of gut health and gastrointestinal diseases (55). The interplay of genetics, lifestyle, and gut flora significantly influences how a person responds to food, making personalized nutrition a comprehensive approach. The gut microbiota's impact extends beyond digestion, affecting various physiological and psychological functions. Profiling the microbiome through modern techniques like metagenomics allows researchers to understand its composition, abundance, and potential health implications. Armed with this microbiome information, healthcare providers can craft personalized nutritional advice, highlighting foods that support good bacteria or those that may disrupt balance (56). Precision nutrition emerges as a powerful tool in modifying the course of gastrointestinal disorders. For individuals with bowel syndrome, tailored dietary strategies based on their unique gut microbiota composition can enhance the effectiveness of interventions like the low-FODMAP diet (57). Similarly, personalized nutrition holds promise in managing IBD by addressing microbial imbalances and identifying specific dietary triggers, leading to reduced inflammation and improved symptom relief. Individuals with food sensitivities can also benefit from precision nutrition by identifying foods that impact their gut microbiota negatively (58). However, despite its potential, precision nutrition faces challenges such as the complexity of microbiome investigation and the need for extensive data interpretation. Direct correlations between the microbiome and health consequences are still under exploration, necessitating further research and development. The shift to precision nutrition for gastrointestinal health represents a departure from conventional approaches, allowing doctors to offer more tailored dietary advice based on an individual's unique microbiota. This approach exemplifies the synergy between advanced science and personalized care, holding the potential to revolutionize our understanding, treatment, and prevention of gastrointestinal disorders.

Clinical indications and therapeutic crescendos

Recent groundbreaking studies highlight the pivotal role of gut microbiota in shaping the future of gastrointestinal health. This expanding knowledge has paved the way for novel therapeutic approaches leveraging the ability to alter the gut microbiome through

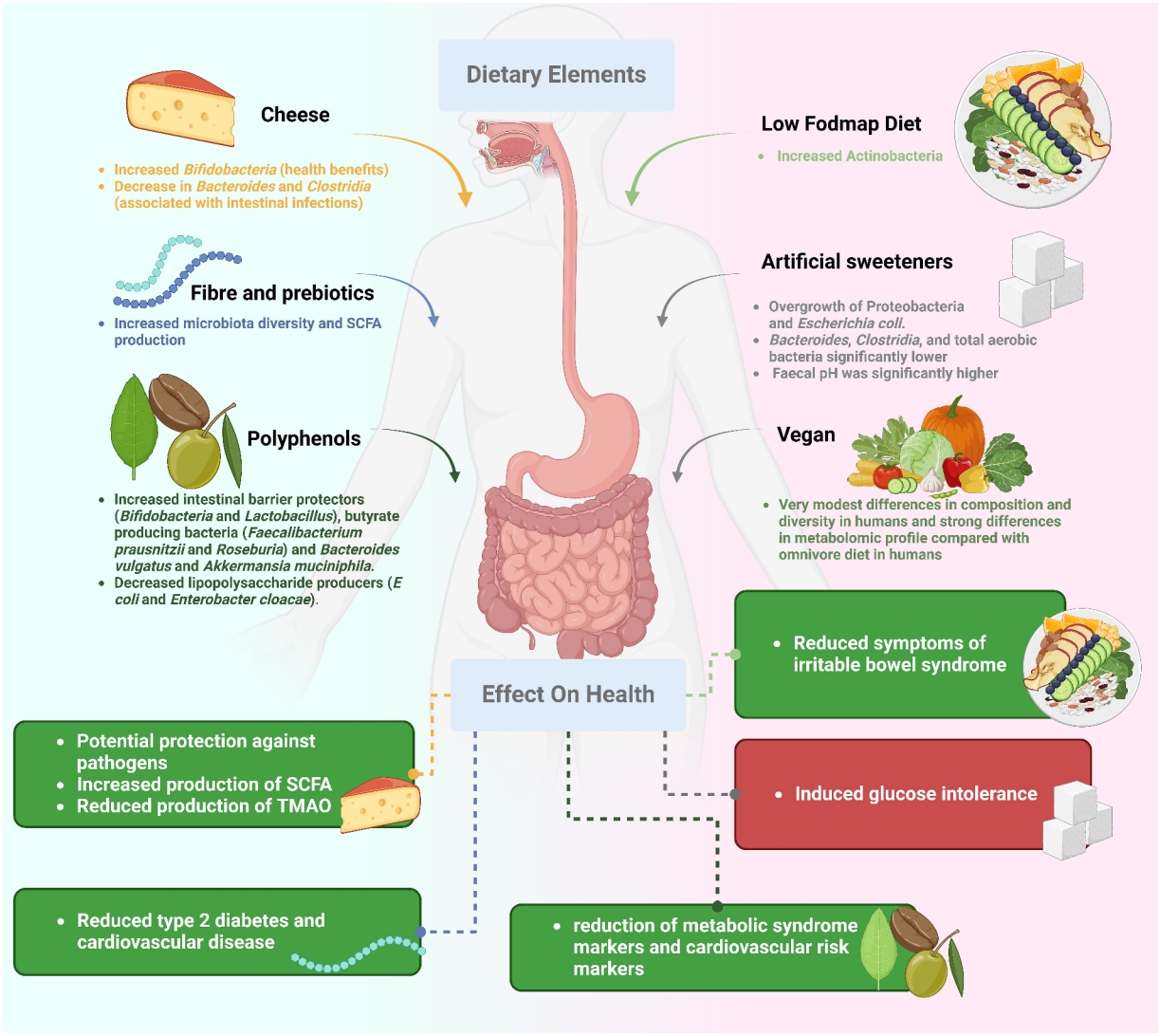

FIGURE 3
Precision nutrition in gastrointestinal health.

dietary changes and microbial therapies. Specific developments in the field include:

Exact probiotics

Moving away from blanket approaches, recent probiotic developments focus on tailoring formulations to address unique microbial imbalances associated with individual illnesses. Probiotics designed to produce specific metabolites or regulate immune responses show promise in treating conditions (59).

Fecal microbiota transplantation

FMT involves transferring feces from a healthy donor to an individual with dysbiotic gut flora. Preliminary results suggest FMT could restore balance in patients with different clinical conditions prompting further investigation into its viability as a treatment (60).

Microbial metabolites

The fermentation products of gut bacteria, known as microbial metabolites, have diverse physiological consequences. Short-chain fatty acids (SCFAs), such as butyrate, are being studied for their anti-inflammatory properties (7).

Nutritional software for individual needs

Technological progress is facilitating the implementation of individualized diet plans, contributing to the precision of dietary interventions (61).

Artificial microorganisms

Scientists are exploring the therapeutic engineering of microbial ecosystems, potentially leading to novel therapies using "designer microbiomes" engineered for specific tasks. Clostridium Cluster XIVa

is under investigation for its potential to reduce inflammation and improve gastrointestinal health (62).

Neuro-gut interventions

Understanding the gut-brain axis opens avenues for new treatments for neurological disorders. Microbiome-targeted interventions, such as modulating neurotransmitters and stimulating the vagus nerve, show promise in addressing conditions like epilepsy and depression. The dynamic world of the gut microbiome's importance to the future of gastrointestinal health is undeniable. Advances in scientific understanding empower us to modify, engineer, and harness its healing potential. The ongoing journey holds promise for groundbreaking therapies that not only treat symptoms but also address microbial imbalances at the root of many gastrointestinal disorders (44, 45). We stand on the brink of a new healthcare era where the microbial inhabitants of our gut become active partners in achieving optimal gut health and overall well-being. This microbial revolution, driven by precision, innovation, and interdisciplinary collaboration, signifies a transformative shift in healthcare toward personalized and effective treatments for gastrointestinal disorders.

Conclusion

The exploration of the intricate landscape of the human microbiome has revealed its profound impact on overall health. From the intricate interplay of nutrients shaping microbial diversity to the promising avenues of precision nutrition, this review underscores the evolving understanding of the gut's pivotal role in human well-being. As we witness the symphony of the gut microbiome, strategies for harmonizing microbial communities through dietary interventions offer tangible solutions for managing gastrointestinal health. Furthermore, the review highlights pioneering clinical approaches, including exact probiotics, fecal microbiota transplantation, and neuro-gut interventions, signaling a transformative shift in healthcare. These advancements not only represent scientific progress but also pave the way for personalized and effective treatments for gastrointestinal disorders. In this symbiotic dance between science and care, the microbial inhabitants of our gut emerge as active collaborators, guiding us toward optimal health. As we stand at the threshold of this new era, the review underscores the importance of precision, innovation, and interdisciplinary collaboration in shaping the future of gastrointestinal health and inspiring avenues for future research and clinical applications.

Author contributions

ZS: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. LP: Methodology, Project administration, Software, Writing – original draft, Writing – review & editing. SP: Conceptualization, Data curation, Investigation, Project administration, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

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The association of composite dietary antioxidant index with periodontitis in NHANES 2009–2014

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Background: To date, evidence is rare regarding whether and how dietary antioxidants are associated with the risk of periodontitis. This study aimed to investigate the association of composite dietary antioxidant index (CDAI) with periodontitis and tooth loss, using data from the National Health and Nutrition Examination Survey (2009–2014).

Methods: A cross-sectional analysis was conducted using data from 10,067 adults aged ≥30 years who underwent assessments of periodontal health and the 1st day dietary recall. Based on a crude model and three adjusted models, multivariate regressions were used to examine the relationship between CDAI and periodontitis-related measurements including probing pocket depth, clinical attachment loss and tooth loss. Subgroup analyses and the restricted cubic splines plots were applied to examine the association between CDAI ingredients and periodontitis.

Results: For the subjects with high CDAI scores, increased CDAI was associated with significant ($P < 0.05$) reduction of severe periodontitis (odd ratio = 0.663, 95% confidence interval: 0.491–0.896) and increased number of remaining teeth (weighted β[SE] = 1.167[0.211]). However, the protective effect of CDAI on periodontitis vanished ($P > 0.05$) in active smokers and former smokers. There were threshold levels for β-carotene, Vitamin A, C and E intakes where the risk of periodontitis significantly decreased ($P < 0.05$) above these levels.

Conclusion: Increased CDAI was associated with reduced risk of periodontitis and tooth loss for non-smokers. It was recommendable that proper dietary intakes of β-carotene, Vitamin A, C and E would be of benefit for preventive dental care and adjuvant therapies for periodontitis.

KEYWORDS

composite dietary antioxidant index, periodontitis, tooth loss, smoking, National Health and Nutrition Examination Survey

1 Introduction

Periodontitis is a progressive chronic inflammatory disease that, when left untreated or inadequately treated, can lead to destruction of the tooth-supporting tissue and ultimately loss of teeth (1). It ranked as the sixth most prevalent disease worldwide, with a prevalence of up to 50% (2, 3). Several factors, including global population growth and aging, have contributed to the increased prevalence of severe periodontitis over the past three decades (2). At the meantime, the global burden of periodontitis has remained substantial and even grown over the years (4).

The pathogenic events in periodontitis involve complex interactions between microbial communities and host responses. The susceptibility to periodontitis varies among different populations (3, 5). Increasing evidence has stressed the predominant role of the host's inflammatory responses in the pathogenesis of periodontitis. Upon microbial challenge, immune cell-mediated oxidative stress propagates the pro-inflammatory signaling and eventually leads to tissue damage (3, 6).

Oxidative stress, characterized by overactivation of reactive oxygen species (ROS) and reduced antioxidant capacity, has been extensively studied as one of the main factors contributing to periodontitis (7). Patients with periodontitis have elevated serum ROS levels, which can be effectively reduced by circulating antioxidant (8). It has been well documented that antioxidant from diet can reduce the body's oxidative stress and lower the risk of systemic diseases, such as diabetes mellitus and cardiovascular diseases (9, 10). These diseases are accompanied by worsened periodontal health and tooth loosening (11). It is plausible to speculate that antioxidant intake from diet might also impact the prevalence and severity of periodontitis. Specifically, in populations and countries with low accessibility to dental care and high prevalence of periodontitis (2), appropriate micronutrient intake may have a significant effect on the morbidity.

To date, evidence is rare regarding the association of dietary antioxidant and the risk of periodontitis and tooth loss. Vanessa et al. demonstrated a positive linear association between the inflammatory degree of the diet (12), measured via the dietary inflammatory index (DII), and periodontitis. Revealing the link between dietary antioxidant intake and periodontitis might have more significance in diet instructions and adjuvant therapeutic strategies. The composite dietary antioxidant index (CDAI) summarized multiple dietary antioxidants including Vitamins A, C, and E, β-carotene, selenium, and zinc (13). This index was developed based on the aggregate effect of these antioxidants. Several researches demonstrated the antioxidant effects of the individual component (14, 15). A previous study demonstrated that CDAI more precisely captures an individual's dietary antioxidant profile and reduces misclassification of exposure (16). In this study, we analyzed the National Health and Nutrition Examination Survey (NHANES) database to investigate the potential association between CDAI and periodontitis, hoping to provide reference for public oral health care based on diet instructions.

2 Materials and method

2.1 Study design and participants

This study used data from the NHANES collected in 2009–2010,2011–2012 and 2013–2014. The data was collected via interviews, physical and laboratory examinations. All of the data in NHANES 2009–2014 were reviewed and approved by the Centers for Disease Control (CDC) and Prevention National Center for Health Statistics Research (NCHS) Ethics Review Board. All of the participants provided a written informed consent. The data derived from edentulous participants or participants with invalid oral examinations or invalid 1st day dietary recalls were excluded.

2.2 Dietary assessment

NHANES participants underwent a dietary recall interview collected in-person followed by a full-mouth periodontal examination for those aged 30 years and older at a mobile examination center. The dietary recall interviews were used to collect data on the types and amounts of foods and beverages (including all types of water) consumed during the last 24 hours (midnight to midnight), and to estimate intakes of nutrients, energy and other ingredients from those foods and beverages. The CDAI is a summary score of multiple dietary antioxidants including Vitamins A, C, and E, β-carotene, selenium, and zinc. In the present study, we calculated the weighted average of the data from the 1st 24-hour recall interviews. Then, we calculated the z-score for each micronutrient parameter. The CDAI was calculated using the formula as below:

$$\mathrm{CDAI} = \sum_{i=1}^{n=6} \frac{\text{Individual Intake} - \text{Mean}}{\text{SE}}$$

For all of the participants, the CDAI scores were equally divided into four grades, CDAI-1st to 4th, in ascending order of the anti-inflammatory capacity.

2.3 Periodontal assessment

Full-month periodontal examination at six sites per tooth was conducted by trained calibrated examiners for participants aged 30 years and older. Participants were eligible for the periodontal assessments if they did not meet any of the health exclusion criteria and had at least one tooth (excluding third molars). Probing pocket depth (PPD) and gingival recession were measured using a HU-Friedy periodontal probe graduated in 2 mm increments. Clinical attachment loss (CAL) was calculated as the difference between PPD and gingival recession (17). The primary outcome was listed as moderate/severe periodontitis according to the recommendations of the Centers for Disease

Control and Prevention (18). The secondary outcomes were the number of remaining teeth (excluding third molars), mean PPD and mean CAL measured at the interproximal sites.

2.4 Covariates

Self-reported socio-demographic characteristics regarding age, gender, race, education level, marital status and family income were collected for each participant. Other data was collected including smoking status, alcohol use, diabetes, hypertension (identified as a self-report of a doctor's diagnosis, systolic blood pressure ≥ 140 mmHg, or diastolic blood pressure ≥ 90 mmHg) (19), hypercholesterolemia. Smoking status were subdivided as never (smoked less than 100 cigarettes in life and not currently smoking), former (smoked at least 100 cigarettes in life and not currently smoking), and active smoker (smoked at least 100 cigarettes in life and currently smoking) (20). Alcohol use was subdivided as never, moderate, heavy, or binge according to definitions from the National Institute on Alcohol Abuse and Alcoholism in the National Institute of Health. Blood examinations were also included as some of the parameters indicated the level of systemic inflammation: white blood cell count (1000 cells/uL), segmented neutrophils number (1000 cell/uL), lymphocyte number (1000 cells/uL), hematocrit(%), platelet count(1000 cell/uL), and total cholesterol (mg/dL) and hemoglobin A1c (Hba1c)(%).

All of the variables listed above were potential confounders that might influence periodontitis. Not all of the participants provided valid data for each of these covariates and there were fewer than 5% missing values. However, direct exclusion may lead to a decrease in sample size and unpredictable bias. Therefore, we used the R package missForest, which is based on the random forest algorithm, for imputation of categorical and continuous variables (21).

2.5 Statistical analysis

Statistical analyses were performed with STATA MP and Rstudio. The level of significance was set at 5%. Individual sample weights were determined using the dietary day one sample weight (WTDRD1) records, to enable extrapolation to the entire noninstitutionalized U.S. population (22). Characteristics grouped by periodontitis status were presented as mean ± SE for continuous variables, and percentage(%) for categorical variables. The baseline characteristics were compared using the weighted linear regression for continuous variables, and the weighted chi-square test for categorical variables (23). Variables with significant association ($P< 0.05$) to periodontitis (Supplementary Table 1) were incorporated into the multivariable analyses (24).

Multivariable logistic regression models were built to evaluate the association between moderate or severe periodontitis (versus no or mild periodontitis), mean PPD, mean CAL, the number of teeth and the CDAI. Three progressively adjusted models were further developed based on the initial crude model. Model 1 was adjusted for age, race, gender, family income and education level; Model 2 was adjusted for covariates in Model 1 plus variables of systemic condition including HbA1c level and hypercholesterolemia. Model 3 was the fully adjusted model, which was adjusted for covariates in Model 2 along with smoking status. To investigate the impact of CDAI on periodontal health in different populations, we conducted a subgroup analysis of the primary outcome. We used the crude and adjusted model (Model 2) to compare between people who were non-smokers, former smokers, or active smokers.

For further investigating the association between the six micronutrients and periodontitis, we used restricted cubic splines (RCS) with 3 knots to flexibly model the association between CDAI and periodontitis, with the abnormal values in each type of micronutrients excluded. In this part, the participants were divided into two groups: no or mild periodontitis, and moderate or severe periodontitis. The association between micronutrient and periodontitis was adjusted according to Model 3. Logistic regression models were also established to explore their linear relationship (Supplementary Table 2).

3 Results

3.1 Characteristics of the study population

Among the 30,468 participants, 20,401 were excluded due to unqualified data either on oral health or the 1st day dietary recall. The flowchart of participants inclusion was shown in Figure 1. Finally, 10,067 participants were included in the present study.

The 10,067 participants were divided into three groups based on the severity of periodontitis. The weighted prevalence was 63.4% for healthy or mild periodontitis, 28.8% for moderate periodontitis, and 7.8% for severe periodontitis. 71.5% of the participants were male in the severe-periodontitis group, while over 50% of the participants were female for the healthy or mild periodontitis group. Compared to the participants with healthy or mild periodontitis, those with moderate or severe periodontitis were more characterized by the features including being older, being Mexican American or non-Hispanic Black, having lower education levels (below

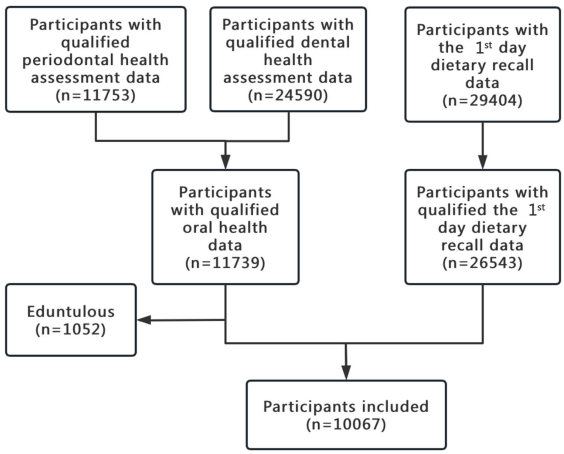

FIGURE 1
The work flow of sample selection from NHANES 2009–2014.

undergraduate), living alone (never having been married, divorced or separated), having a lower household income (under $55,000 per year), being smokers or drinkers, or having lower CDAI (Table 1).

3.2 Association of the CDAI and periodontal outcomes

Table 2 showed the association between CDAI and periodontal outcomes. In the unadjusted multivariable logistic regression model, a higher CDAI was associated with decreased prevalence of moderate and severe periodontitis, increased number of remaining teeth, reduced PPD and CAL. In comparison to the CDAI-1st group, the CDAI-4th group had significantly lower risk of moderate (odd ratio [OR] = 0.747, 95% confidence interval [CI]: 0.63–0.886, P = 0.001) and severe (OR = 0.712, 95% CI: 0.54–0.939, P = 0.016) periodontitis according to the non-adjusted model, with the risks reduced by 25.3% and 28.8%, respectively. After adjustments for the characteristics of demographics and systemic conditions in Model 2, the multivariable regression analysis still demonstrated a significant association between CDAI and severe periodontitis (OR = 0.663, 95% CI: 0.491–0.896, P = 0.008) in the CDAI-4th group. The association between PPD, CAL and the CDAI was similar to that between periodontitis and CDAI. Furthermore, we found that the CDAI showed a stronger association with CAL than with PPD across all the four models. The CDAI was also significantly and positively correlated with the number of remaining teeth.

3.3 Smoking status had impact on the association between CDAI and periodontal outcomes

When smoking was included as a covariate in the model, as mentioned in Model 3, the protective effect of CDAI on periodontitis became ambiguous. However, the coefficient of logistic regression changed by approximately 10% when compared with that in Model 2. And multifactorial analysis revealed that smoking status had a significant effect on periodontitis (Supplementary Table 1). Therefore, we performed subgroup analyses to explore the potential influence of smoking status on the association between CDAI and periodontitis.

As shown in Table 3, CDAI lost the protective effect on periodontitis in active smokers, and even in the former smokers (P > 0.05). Among the non-smokers, the risk of severe periodontitis for the CDAI-4th group reduced by 56.6% (OR = 0.434, 95% CI: 0.291–0.649, P< 0.001), compared with the CDAI-1st group. Similar results were found for moderate periodontitis, with the risk reduced by 34.2% (OR = 0.658, 95% CI: 0.523–0.829, P< 0.001). The impact of smoking on the periodontal protective role of CDAI was further investigated by the model adjusted for all of the characteristics except smoking status (Model 2). In this model, the CDAI showed protective effect only among non-smokers for severe periodontitis (OR = 0.458, 95% CI: 0.289–0.728, P = 0.001).

Similar results were found regarding the association of CDAI with mean PPD and mean CAL in Model 2. For the non-smokers in the CDAI-4th group, the CDAI were significantly associated with PPD values (weighted β[SE] = -0.089[0.026], P< 0.001) and CAL values (weighted β[SE] = -0.126[0.031], P< 0.001). For the active smokers or former smokers, there were no correlations (P > 0.05) between PPD or CAL and CDAI. Additionally, higher CDAI were significantly associated (weighted β[SE]=1.639[0.517], P = 0.002) with increased number of remaining teeth among the former smokers.

3.4 Non-linear association between the CDAI ingredients and periodontitis

Each ingredient of the CDAI and its linear correlation with periodontitis were investigated using multivariate linear regression. Vitamin A, Vitamin E and β-carotene were found to be significantly associated with periodontitis (P< 0.05) in the fully adjusted model (Supplementary Table 2). The association between the six micronutrients and periodontitis was shown in the restricted cubic splines curves (Figure 2). The association of Vitamin A and Vitamin E with periodontitis assumed a L-shaped relationship (Figures 2A, C, P for non-linearity< 0.05), while Vitamin C and β-carotene had a U-shaped relationship with periodontitis (Figures 2B, F, P for non-linearity< 0.05).

We found that there were threshold levels for β-carotene, Vitamin A, C and E intakes where the risk of periodontitis significantly increased below these levels and was inversely correlated with the amount of micronutrient intakes. For Vitamin A and E, dietary intakes above the threshold levels were associated with steadily protective effect on periodontal health, within the dose range observed in this study. However, having Vitamin C and β-carotene far beyond the threshold levels tended to lead to increased risk of periodontitis. No significant association (P > 0.05) was observed between selenium or zinc with periodontitis.

4 Discussion

In light of this study, independent of factors including age, gender, race, education level, family income, living habit and systemic conditions, the stratified CDAI was significantly associated with periodontitis and tooth loss. However, the periodontal protective role of CDAI vanished for active and former smokers.

The CDAI ingredients contain essential trace elements for various proteins and enzymes that are involved in antioxidant and anti-inflammatory processes, collagen synthesis, tyrosine metabolism, and protection against cancers (16, 25). Despite the widely recognized effectiveness and accessibility of the micronutrient protecting against inflammatory diseases (15), previous studies on the associations of nutrients with the risk of periodontitis have yielded controversial findings (26–31). The discrepancies might have arisen from 1) the interactions between

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TABLE 1 Characteristics of the participants.

Characteristics	Overall	No/ Mild Periodontitis	Moderate Periodontitis	Severe Periodontitis	*P* value
Number	10,067	5,597	3,367	1,103	
Weighted Number	441,400,000 (100.0%)	280,000,000(63.4%)	127,000,000(28.8%)	34,400,000(7.8%)	
Continuous variables, mean ± SE					
White Blood Cell (1000 cell/uL)	7.126 ± 0.029	6.977 ± 0.036	7.294 ± 0.054	7.716 ± 0.119	<0.001**
Age (year)	50.9740.169	48.565 ± 0.215	55.317 ± 0.301	54.545 ± 0.476	<0.001**
Lymphocyte Number (1000 cells/uL)	2.0880.01	2.069 ± 0.012	2.115 ± 0.019	2.145 ± 0.034	0.003*
Segmented Neutrophils (1000 cell/uL)	4.2470.023	4.139 ± 0.028	4.364 ± 0.043	4.695 ± 0.099	<0.001**
Hematocrit (%)	41.390.055	41.214 ± 0.071	41.532 ± 0.101	42.301 ± 0.179	<0.001**
Platelet (1000 cell/uL)	237.2520.823	238.258 ± 1.021	235.204 ± 1.512	236.63 ± 3.382	0.068
HbA1c (%)	5.6830.011	5.562 ± 0.012	5.868 ± 0.023	5.995 ± 0.053	<0.001**
Total Cholesterol (mg/dL)	5.150.015	5.161 ± 0.02	5.138 ± 0.027	5.101 ± 0.05	0.256
Tooth Number	24.6030.077	25.755 ± 0.082	22.899 ± 0.162	21.525 ± 0.321	<0.001**
Clinical Attachment Loss (mm)	1.6920.013	1.248 ± 0.01	2.12 ± 0.02	3.722 ± 0.064	<0.001**
Probing Pocket Depth (mm)	1.6440.008	1.389 ± 0.007	1.88 ± 0.013	2.842 ± 0.035	<0.001**
Categorical variables, percentage					
Gender (female)	0.512	0.567	0.451	0.285	<0.001**
Race					<0.001**
Mexican American	0.080	0.061	0.110	0.127	
Other Hispanic	0.054	0.051	0.058	0.060	
Non-Hispanic White	0.689	0.740	0.621	0.522	
Non-Hispanic Black	0.107	0.083	0.131	0.208	
Other Race	0.071	0.065	0.080	0.083	
Education Level (college or above)	0.641	0.720	0.536	0.391	<0.001**
Marital Status (live with someone)	0.685	0.717	0.637	0.601	<0.001**
High Income (more than $54,999 per year)	0.559	0.648	0.418	0.353	<0.001**
Alcohol Use					<0.001**
Never	0.127	0.117	0.155	0.111	
Moderate	0.402	0.436	0.360	0.287	
Heavy	0.236	0.264	0.192	0.180	
Binge	0.234	0.184	0.293	0.422	
Smoking Status					<0.001**
Never	0.557	0.628	0.458	0.345	
Former Smoker	0.267	0.250	0.302	0.276	
Active Smoker	0.176	0.123	0.239	0.378	
Diabetes					<0.001**
No	0.830	0.859	0.772	0.809	
Prediabetes	0.076	0.073	0.087	0.056	

(Continued)

TABLE 1 Continued

Characteristics	Overall	No/ Mild Periodontitis	Moderate Periodontitis	Severe Periodontitis	*P* value
Categorical variables, percentage					
Yes	0.094	0.068	0.141	0.134	
Have Hypertension	0.408	0.359	0.488	0.502	<0.001**
Have Hypercholesterolemia	0.403	0.386	0.456	0.350	<0.001**
CDAI					<0.001**
1st	0.250	0.233	0.279	0.284	
2nd	0.250	0.248	0.249	0.267	
3rd	0.250	0.259	0.238	0.223	
4th	0.250	0.260	0.233	0.226	

*Indicates *P* value< 0.05; **indicates *P* value< 0.001. SE, Standard Error; HbA1c, glycated hemoglobin A1c; CDAI, composite dietary antioxidant index.

TABLE 2 The risk of periodontitis for the four CDAI groups indicated by the four models (N =10,067).

		Non-Adjusted	Model 1	Model 2	Model 3
Periodontitis					
Moderate	CDAI-1st	Reference	Reference	Reference	Reference
	CDAI-2nd	0.839 (0.712, 0.991)0.039*	0.872 (0.728, 1.045)0.138	0.871 (0.726, 1.044)0.136	0.918 (0.763, 1.103)0.361
	CDAI-3rd	0.768 (0.649, 0.908)0.002*	0.82 (0.682, 0.985)0.034*	0.82 (0.682, 0.986)0.035*	0.864 (0.717, 1.041)0.125
	CDAI-4th	0.747 (0.63, 0.886)0.001*	0.792 (0.654, 0.96)0.017*	0.787 (0.65, 0.955)0.015*	0.845 (0.697, 1.024)0.086
Severe	CDAI-1st	Reference	Reference	Reference	Reference
	CDAI-2nd	0.884 (0.687, 1.137)0.335	0.922 (0.7, 1.215)0.563	0.915 (0.694, 1.207)0.531	0.995 (0.747, 1.324)0.970
	CDAI-3rd	0.707 (0.546, 0.915)0.008*	0.738 (0.555, 0.979)0.035*	0.731 (0.55, 0.969)0.030*	0.788 (0.587, 1.059)0.114
	CDAI-4th	0.712 (0.54, 0.939)0.016*	0.677 (0.499, 0.918)0.012*	0.663 (0.491, 0.896)0.008*	0.728 (0.534, 0.992)0.044*
Number of Teeth					
CDAI-1st		Reference	Reference	Reference	Reference
CDAI-2nd		1.281 (0.831, 1.731)<0.001**	0.878 (0.469, 1.286)<0.001**	0.872 (0.464, 1.279)<0.001**	0.741 (0.339, 1.143)<0.001**
CDAI-3rd		1.796 (1.349, 2.242)<0.001**	1.077 (0.668, 1.487)<0.001**	1.059 (0.65, 1.469)<0.001**	0.912 (0.508, 1.316)<0.001**
CDAI-4th		2.16 (1.728, 2.592)<0.001**	1.171 (0.758, 1.584)<0.001**	1.167 (0.754, 1.58)<0.001**	0.979 (0.573, 1.385)<0.001**
PPD					
CDAI-1st		Reference	Reference	Reference	Reference
CDAI-2nd		-0.053 (-0.098, -0.008)0.022*	-0.031 (-0.073, 0.011)0.150	-0.032 (-0.073, 0.01)0.141	-0.016 (-0.058, 0.026)0.449
CDAI-3rd		-0.07 (-0.115, -0.025)0.002*	-0.044 (-0.086, -0.001)0.043*	-0.045 (-0.087, -0.003)0.035*	-0.027 (-0.069, 0.015)0.204
CDAI-4th		-0.07 (-0.114, -0.025)0.002*	-0.075 (-0.118, -0.032)0.001*	-0.076 (-0.119, -0.033)< 0.001**	-0.054 (-0.096, -0.012)0.011*
CAL					
CDAI-1st		Reference	Reference	Reference	Reference
CDAI-2nd		-0.129 (-0.206, -0.052)0.001*	-0.091 (-0.16, -0.022)0.010*	-0.091 (-0.16, -0.022)0.010*	-0.059 (-0.127,0.01)0.094
CDAI-3rd		-0.178 (-0.254, -0.102)<0.001**	-0.115 (-0.183, -0.047)0.001*	-0.115 (-0.183, -0.047)0.001*	-0.077 (-0.144, -0.011)0.023*
CDAI-4th		-0.194 (-0.265, -0.123)<0.001**	-0.154 (-0.223, -0.086)<0.001**	-0.156 (-0.224, -0.088)<0.001**	-0.109 (-0.175, -0.043)0.001*

*Indicates *P* value< 0.05; **indicates *P* value< 0.001. CDAI, composite dietary antioxidant index; PPD, probing pocket depth; CAL, clinical attachment loss. Model 1 adjusted for age, race, gender, family income and education level; and Model 2 adjusted for age, race, gender, family income, education level, HbA1c level and hypercholesterolemia; and Model 3 adjusted for age, race, gender, family income, education level, HbA1c level, hypercholesterolemia and smoking status.

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TABLE 3 Multi-regression analysis of the association between CDAI and periodontal outcomes stratified according to smoking status.

		Never Smoker		Former Smoker		Active Smoker	
		Non-Adjusted	Adjusted	Non-Adjusted	Adjusted	Non-Adjusted	Adjusted
Periodontitis							
Moderate	CDAI-1st	Reference	Reference	Reference	Reference	Reference	Reference
	CDAI-2nd	0.891 (0.717,1.108)0.302	1.014 (0.797,1.289)0.910	1.007 (0.717,1.415)0.967	0.992 (0.687,1.432)0.964	0.713 (0.481,1.058)0.092	0.694 (0.449,1.071)0.099
	CDAI-3rd	0.66 (0.523,0.832)<0.001**	0.796 (0.615,1.03)0.084	1.103 (0.795,1.531)0.558	1.127 (0.791,1.606)0.508	0.868 (0.581,1.298)0.491	0.78 (0.504,1.208)0.266
	CDAI-4th	0.658 (0.523,0.829)<0.001**	0.79 (0.607,1.027)0.078	0.902 (0.644,1.262)0.548	0.962 (0.664,1.392)0.835	1.184 (0.794,1.766)0.408	1.005 (0.646,1.565)0.982
Severe	CDAI-1st	Reference	Reference	Reference	Reference	Reference	Reference
	CDAI-2nd	0.753 (0.511,1.108)0.151	0.85 (0.553,1.305)0.456	0.814 (0.501,1.323)0.407	0.81 (0.473,1.385)0.442	1.401 (0.867,2.266)0.169	1.323 (0.766,2.286)0.316
	CDAI-3rd	0.609 (0.408,0.908)0.015*	0.706 (0.451,1.105)0.128	0.748 (0.446,1.252)0.269	0.777 (0.446,1.355)0.374	1.138 (0.694,1.863)0.609	0.9 (0.506,1.603)0.722
	CDAI-4th	0.434 (0.291,0.649)<0.001**	0.458 (0.289,0.728)0.001*	0.766 (0.421,1.395)0.384	0.742 (0.396,1.394)0.354	1.772 (1.1,2.855)0.019*	1.252 (0.738,2.125)0.404
Number of Teeth							
CDAI-1st		Reference	Reference	Reference	Reference	Reference	Reference
CDAI-2nd		1.049 (0.569,1.53)<0.001**	0.574 (0.143,1.004)0.009*	1.209 (0.065,2.352)0.038*	0.944 (-0.087,1.976)0.073	1.516 (0.459,2.573)0.005*	1.061 (0.083,2.039)0.034*
CDAI-3rd		1.715 (1.243,2.186)<0.001**	0.818 (0.382,1.254)<0.001**	2.087 (1.075,3.099) <0.001**	1.563 (0.63,2.495)0.001*	0.822 (-0.512,2.156)0.227	0.34 (-0.813,1.493)0.563
CDAI-4th		2.024 (1.595,2.454)<0.001**	0.852 (0.444,1.259)<0.001**	2.683 (1.618,3.748) <0.001**	1.639 (0.626,2.652)0.002*	0.896 (-0.312,2.105)0.146	0.465 (-0.663,1.592)0.419
PPD							
CDAI-1st		Reference	Reference	Reference	Reference	Reference	Reference
CDAI-2nd		-0.036 (-0.091,0.019)0.199	-0.012 (-0.063,0.039)0.653	-0.033 (-0.125,0.059)0.482	-0.032 (-0.119,0.054)0.461	-0.014 (-0.136,0.109)0.826	-0.022 (-0.136,0.092)0.702
CDAI-3rd		-0.074 (-0.127, -0.021)0.007*	-0.038 (-0.088,0.013)0.142	-0.038 (-0.128,0.052)0.404	-0.04 (-0.126,0.046)0.364	0.048 (-0.078,0.175)0.451	0.009 (-0.114,0.131)0.887
CDAI-4th		-0.1 (-0.151, -0.049)<0.001**	-0.089 (-0.139, -0.039)<0.001**	-0.023 (-0.116,0.07)0.623	-0.042 (-0.133,0.048)0.360	0.122 (0.002,0.242)0.047*	0.022 (-0.091,0.135)0.706
CAL							
CDAI-1st		Reference	Reference	Reference	Reference	Reference	Reference
CDAI-2nd		-0.094 (-0.17, -0.019)0.015*	-0.042 (-0.109,0.025)0.222	-0.114 (-0.3,0.072)0.230	-0.105 (-0.275,0.065)0.226	-0.058 (-0.278,0.162)0.606	-0.066 (-0.263,0.131)0.513
CDAI-3rd		-0.188 (-0.256, -0.12)<0.001**	-0.094 (-0.154, -0.034)0.002*	-0.15 (-0.322,0.023)0.088	-0.123 (-0.282,0.036)0.129	0.061 (-0.184,0.305)0.626	-0.015 (-0.236,0.206)0.896
CDAI-4th		-0.203 (-0.269, -0.137)<0.001**	-0.126 (-0.189, -0.064)<0.001**	-0.189 (-0.354, -0.024)0.025*	-0.134 (-0.293,0.025)0.099	0.095 (-0.12,0.311)0.387	-0.074 (-0.272,0.124)0.465

*Indicates *P* value< 0.05; **indicates *P* value< 0.001. CDAI, composite dietary antioxidant index; PPD, probing pocket depth; CAL, clinical attachment loss. Adjusted model, namely Model 3, adjusted for age, race, gender, family income, education level, HbA1c level, hypercholesterolemia and smoking status.

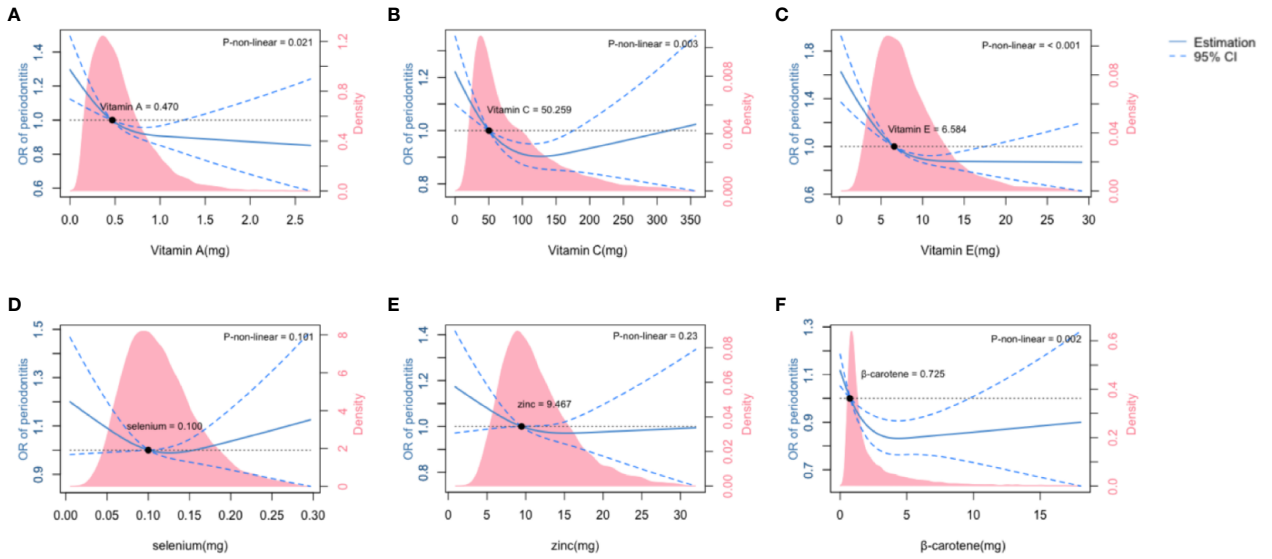

FIGURE 2
The association of six ingredients of Composite Diatery Antioxidant Index (CDAI) with moderate and severe periodontitis. The association of Vitamin A **(A)**, Vitamin C **(B)**, Vitamin E **(C)**, Selenium **(D)**, Zinc **(E)** and β-carotene **(F)** with the OR of periodontitis was shown in the restricted cubic splines, as indicated by the model adjusted for age,race,gender,family income, education level, HbA1c level, hypercholesterolemia and smoking status. The dashed line represents the 95%CI. Density plots represent the distribution of populations with consecutively varied levels of micronutrient intakes. CI, confidence interval; OR, odds ratio.

different nutrients being overlooked when each type of nutrient was considered separately; 2) some mild effects of the nutrients being overwhelmed by stronger influence from specific covariates (30); 3) non-linear association being underestimated in a large population (32); 4) difficulty in recognizing slight impact of micronutrient levels in the serum on periodontal disease (29). In this study, we evaluated the anti-oxidant capacity of micronutrient through dietary intake using CDAI, and investigated the impact of CDAI on periodontitis, attempting to draw a more reliable conclusion by avoiding the above defects.

Interestingly, the present study revealed stronger association of the CDAI with CAL than with PPD. Pathologically, with CAL and PPD both being the essential elements for the diagnosis of periodontitis, CAL is a more direct indicator of destruction of tooth supporting tissue including alveolar bone (33). The strong link between CDAI and CAL stressed the potential protective effect of CDAI on periodontal tissue. In the fully adjusted model (Model 3), despite vanished effect of CDAI on periodontitis-related factors including CAL and PPD, the stratified CDAI still significantly correlated with the number of remaining teeth. Since tooth loss may result from multiple causes such as caries and periodontal diseases (34), the beneficial effect of micronutrient on teeth may be derived from protection against tooth decay, as well as factors other than periodontal health (35).

It is well documented that conventional and electronic cigarette smoking are prominent risk factors for periodontitis (36, 37). Cigarette smoke contains toxic substances that dissolve in oral epithelial linings and spread throughout the body, interfering in biological events involved in redox homeostasis, immune responses, bone metabolism and tissue repair (38, 39). Tobacco-rich conditions facilitate proliferation of *Filifactor alocis* and enhance its pathogenicity (40). The detrimental effect of smoking on periodontal health persists long even after quitting. Former smokers still had a higher risk of tooth loss caused by periodontal diseases compared to the average population, and it took up to 15 years after quitting to reach the same risk level as before smoking (41). Our study lent support to this notion by demonstrating periodontal protective effect of CDAI on non-smokers but not on active or former smokers.

Previous studies had demonstrated the non-linear relations between CDAI and oxidative stress-related diseases (23, 42). The association between specific micronutrients and periodontitis was examined by using two-piecewise linear regression models (31). In the present study, we focused on dietary micronutrient intakes and looked into the six CDAI ingredients, based on models adjusted for covariates including demographics, lifestyles and systemic conditions. It is worth noting that there were threshold levels for the CDAI ingredients, having micronutrients below which was associated with increased risk of periodontitis. These threshold levels were 0.470 mg for Vitamin A, 6.584 mg for Vitamin E, 50.259 mg for Vitamin C, and 0.725 mg for β-carotene. By superimposing the density plots on the restricted cubic splines (Figure 2), we revealed that there were large populations whose intakes of Vitamin A, C and E were lower than the threshold levels. According to the UK National Health Service, the recommended dietary allowances (RDA) for Vitamin A, C and E were 0.6~0.7 mg, 40 mg and 3~4 mg, respectively (15). Our study confirmed the validity of these RDAs in protecting periodontal health, and yielded further suggestions that slightly higher intakes of Vitamin C and E above the RDAs might be recommended for patients accepting adjuvant therapies, and for preventive dental care for the populations at high risk of periodontitis.

Note that Vitamin intakes were not the more the better. The risk of periodontitis tended to increase again after high intakes of Vitamin C and β-carotene far beyond the threshold levels, possibly due to overdosed effect or imbalanced nutrition (43). Although with not-yet-observed overdosed effect of Vitamin A and E on periodontitis, it is of vital importance to take into consideration the whole body when making diet instructions. Overabundance of Vitamin A was known to cause liver toxicity (15).

The role of zinc and selenium on antioxidation and periodontal regeneration have been reported in previous studies (15, 44). Several studies on animal models revealed their effectiveness in ameliorating periodontitis (45, 46). However, this study showed that zinc or selenium had no significant association with the risk of periodontitis. The discrepancy might result from the differences in the delivery route (drug administration versus dietary intakes), the form of drug or food (a single drug versus a single element separately analyzed from composite nutrients), and sample selection (diseased subjects versus general populations). A previous study showed that serum zinc levels were associated with the risk of periodontitis in non-diabetic smokers but not non-smokers (47), suggesting the periodontal protective effect of zinc in specific but not general conditions. Further prospective studies like randomized controlled trails are warranted to determine the effect of specific micronutrients from dietary intakes.

The essential role of oxidative stress in pathogenesis of periodontitis has been well recognized. Free radicals contribute to tissue destruction by damaging DNA and proteins, acting as intracellular signal mediators for osteoclast activation, and causing lipid peroxidation and bone destruction (1). Antioxidants protect against oxidative stress by eliminating excessive free radicals (48). Studies have demonstrated that the components of CDAI contributed to the decrease in oxidative stress (14, 15), suggesting that the CDAI components might help strengthen systemic antioxidant defense mechanisms. Interestingly, Hung N. Luu et al. (49) presented a different conclusion that CDAI was not significantly correlated to oxidative stress. The discrepancy of the results may be caused by differences in samples and evaluation metrics. Luu's study focused on the Chinese population, samples with different ethnic background from the United States. Additionally, in Luu's study oxidative stress levels were measured using Urinary F2 isoprostanes and Urinary F2 isoprostane metabolites, with no consideration of indicators like malondialdehyde, glutathione, glutathione reductase etc.

Our study is the first to investigate the collective effect of dietary antioxidant intakes on periodontal health based on a large population. The NHANES used a multi-stage probability sampling process to create samples that were well representative of the noninstitutionalized population in the United States. Thus, the conclusion of this study should be applicable to the general population in the United States. Although with significant association, the cause-and-effect relationship between CDAI and periodontitis cannot be determined by this cross-sectional study. However, the effectiveness of a micronutrient-assisted nonsurgical therapy for chronic periodontitis proved by a randomized, double-blind trial (50) suggested the causal effect of micronutrient intakes on improvement of periodontal health, which was less likely to be explained by the reverse causality.

Considering the individual variability in susceptibility to periodontitis and regional difference in accessibility to dental care across various populations (51), incorporating dietary antioxidant intake into long-term prevention and adjuvant therapy may be a cost-effective and sustainable approach. In light of this study, we recommend proper micronutrient intake from food for adults. The collective effect of multiple antioxidants in a diet helps protect against periodontitis. Moreover, considering the etiological association between periodontitis and systemic diseases such as diabetes, Alzheimer's disease and pre-eclampsia (52–54), dietary antioxidant intakes may also be beneficial for periodontitis patients who have elevated risks of developing these non-communicable diseases.

Although with interesting findings, the results of this study should be interpreted with caution. The pathogenesis of periodontitis is complicated. The effect of nutrients on periodontal health status may be direct or indirectly caused by the nutrient-correlated factors like dietary patterns, or diet-modulated general conditions like systemic diseases. Additionally, 24 hours are not long enough to give an overall and accurate reflection of individual's dietary habit. The dietary intake record in a 24-hour timeframe is just a representative, rather than general dietary status. It is difficult and practically impossible to include all of the confounding factors during surveys. Some possible covariates like pregnancy, medications, lifestyles, oral hygiene status and systemic diseases other than what we have already covered, were of limited availability in the database. Further studies would be of interest after acquisition of extended information.

5 Conclusions

Our study showed that increased CDAI was associated with reduced risk of periodontitis and tooth loss. There were threshold levels for β-carotene, Vitamin A, C and E where dietary intakes above these levels were associated with better periodontal health. However, the protective effects were largely reduced and even eliminated in subjects with smoking history or current smoking status.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the [patients/participants OR patients/participants legal guardian/next of kin] was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

ZM: Methodology, Formal analysis, Writing – original draft, Writing – review & editing. WZ: Data curation, Writing – original draft, Writing – review & editing. XM: Software, Writing – review & editing. HX: Conceptualization, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2024.1384272/full#supplementary-material

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Association between dietary intake of niacin and stroke in the US residents: evidence from national health and nutrition examination survey (NHANES) 1999–2018

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Objective: This study aims to explore the association between niacin intake and stroke within a diverse, multi-ethnic population.

Methods: A stringent set of inclusion and exclusion criteria led to the enrollment of 39,721 participants from the National Health and Nutrition Examination Survey (NHANES). Two interviews were conducted to recall dietary intake, and the USDA's Food and Nutrient Database for Dietary Studies (FNDDS) was utilized to calculate niacin intake based on dietary recall results. Weighted multivariate logistic regression was employed to examine the correlation between niacin and stroke, with a simultaneous exploration of potential nonlinear relationships using restricted cubic spline (RCS) regression.

Results: A comprehensive analysis of baseline data revealed that patients with stroke history had lower niacin intake levels. Both RCS analysis and multivariate logistic regression indicated a negative nonlinear association between niacin intake and stroke. The dose-response relationship exhibited a non-linear pattern within the range of dietary niacin intake. Prior to the inflection point (21.8 mg) in the non-linear correlation between niacin intake and stroke risk, there exists a marked decline in the risk of stroke as niacin intake increases. Following the inflection point, the deceleration in the decreasing trend of stroke risk with increasing niacin intake becomes evident. The inflection points exhibit variations across diverse populations.

Conclusion: This investigation establishes a negative nonlinear association between niacin intake and stroke in the broader American population.

KEYWORDS

niacin, stroke, NHANES, cross-sectional study, RCS

Introduction

Niacin, also known as Vitamin B3, is a water-soluble vitamin with various benefits for human health (1). Niacin can be obtained through various foods such as fish, nuts, whole grains, and can also be supplemented through supplements. As widely known, niacin plays a positive role in maintaining skin health and helps prevent skin issues such as dermatitis and dryness (2). As an antioxidant, niacin helps combat damage caused by free radicals, thereby contributing to slowing down the aging process (3). Existing studies have shown that niacin participates in the energy metabolism process within the body, aiding in the conversion of food into usable energy, and is crucial for maintaining normal metabolism (4–6). Besides, niacin helps regulate cholesterol levels in the blood, particularly by lowering low-density lipoprotein cholesterol (LDL-C), promoting cardiovascular health (7). However, the association of dietary intake of niacin and the prevalence of stroke still remains unclear.

Globally, stroke is one of the major causes of death and disability (8–10). According to data from the World Health Organization (WHO), there are over 15,000,000 new cases of stroke worldwide each year. In developed countries, the incidence and mortality rates of stroke are relatively lower due to improved healthcare conditions and lifestyles (8, 11). However, in some developing countries, the incidence of stroke may be higher due to factors such as poor lifestyle choices, hypertension, diabetes, and other risk factors (12–14). Dyslipidemia is one of the important risk factors for stroke, as it can lead to the formation of atherosclerosis, narrowing the blood vessel walls and making them prone to thrombosis, thereby increasing the risk of stroke (15–17). Particularly, elevated LDL-C levels are considered a major driving factor for the development of atherosclerosis (18, 19). Numerous studies have demonstrated that Vitamin B3 has significant neuroprotective effects, including the enhancement of vascular function, reduction of oxidative stress, and improvement of lipid profiles. For instance, research by Cui et al. showed that Vitamin B3 supplementation reduced the incidence of ischemic strokes in animal models by promoting angiogenesis and neuronal survival (20). Additionally, clinical studies such as those by Teo et al. (21) have indicated that higher dietary intake of niacin is associated with a lower risk of stroke in human populations (21). These findings highlight the potential of niacin as a preventive measure against stroke, supporting its relevance in our study.

Due to its potential benefits in reducing LDL-C, increasing HDL-C levels, decreasing triglyceride levels, and improving overall lipid profile, niacin may lower the risk of stroke by reducing lipid metabolism abnormalities (20, 21). Niacin can also reduce oxidative stress levels, maintain endothelial function, and decrease vascular wall damage, thereby lowering the risk of atherosclerosis and thrombosis formation (22). These benefits contribute to maintaining vascular health, thus aiding in the prevention of stroke. Currently, there is insufficient scientific evidence to suggest that a significant reduction in the occurrence of stroke can be achieved by appropriately increasing niacin intake. Although niacin has shown regulatory effects on lipid metabolism and oxidative stress in some studies, its exact effectiveness in stroke prevention still requires further research for confirmation.

In the present study, we enrolled eligible participants from NHANES database and conducted a cross-sectional analysis to explore the relationship between dietary intake of niacin and the prevalence of stroke in a large normal population.

Methods

Study population

NHANES is a nationally representative survey conducted throughout the United States, selecting participants through random sampling to ensure representative data for the entire U.S. population. It is implemented in two-year cycles. Each cycle involves face-to-face interviews, physical examinations, laboratory tests, and other health measurements conducted on thousands of residents (23). The data from NHANES are widely utilized in shaping public health policies, guiding research, and evaluating national health objectives. Researchers, policymakers, and the public have access to NHANES' database to obtain information about the health and nutritional status of the U.S. population. We initially included 101,316 participants in the present study. Exclusion criteria as follows: (1) participants aged below 18 or above 80 years (n = 46,369); (2) participants with no dietary data, or missing niacin intake data (n = 10,535); (3) pregnant participants (n = 1,413); (4) individuals lacking stroke status (n = 3,278). Eventually, a total of 39721 participants were ultimately included. The flowchart of recruitment process can be found in Figure 1.

Assessment of dietary intake of niacin

Dietary intake of niacin was obtained through interview, also known as What We Eat in America (WWEIA). The US Department of Agriculture (USDA) and the US Department of Health and Human Services (DHHS) collaboratively carried out the interview. All eligible NHANES participants undergo two 24-hour dietary recall interviews to disclose the types and amounts of foods they consumed in the 24 hours prior to the interview (from midnight to midnight). The initial dietary recall takes place in person at the Mobile Examination Center (MEC), and the subsequent recall is conducted via a phone interview around 3 to 10 days later. To calculate the nutrients and food components in various food items, the USDA's Food and Nutrient Database for Dietary Studies (FNDDS) is utilized (24). The dataset, which encompasses the overall nutrient intakes, acts as a brief documentation of each individual's nutrient intake. For this research, the participants' daily niacin intake is established by computing the average of their two dietary recalls.

Assessment of stroke

Stroke identification in this study relied on individuals disclosing a previous diagnosis from a medical practitioner during in-person interviews. Those who answered positively to the question, "Have you ever been told by a doctor or healthcare provider that you had a stroke?" were considered to have history

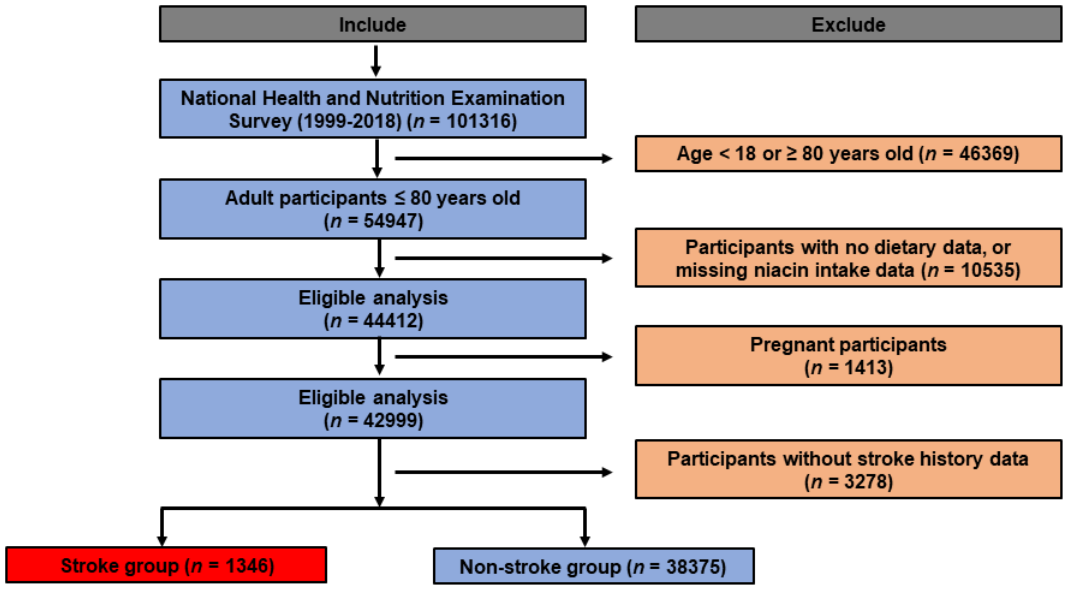

FIGURE 1
Participants enrollment flowchart.

of stroke. It's essential to acknowledge that using self-reported data can be influenced by memory bias, potentially impacting how the information is interpreted (25). Furthermore, while the NHANES database lacks specific details about the stroke types, it's reasonable to assume that a significant portion of participants identified as stroke cases likely had ischemic strokes.

Covariates

Demographic data were obtained using standardized surveys that covered gender, race/ethnicity, educational background, smoking habits, and alcohol consumption. Alcohol consumption was defined as having consumed at least 12 drinks in the year before the survey. Body mass index (BMI) was utilized to assess overweight and obesity, with values exceeding 25 and 30 indicating overweight and obesity, respectively (26). Trained clinicians measured systolic/diastolic blood pressure (SBP/DBP), and the final blood pressure reading was calculated as the average of three consecutive readings taken at half-minute intervals. Laboratory tests, conducted following standardized procedures, determined various parameters. Participants meeting any of the following criteria were classified as having hypertension: (1) Average systolic blood pressure (SBP) $\geq$ 140 mmHg; (2) Average diastolic blood pressure (DBP) $\geq$ 90 mmHg; (3) Self-reported hypertension diagnosis; (4) Current use of antihypertensive medications (27). Individuals with a previous diagnosis of diabetes by a physician or health professional were categorized as having diagnosed diabetes (28–30).

Statistical methods

Due to the complex sampling methods employed in the NHANES survey, our analytical approaches incorporated sample weights customized for specific research periods to ensure accurate calculations of health-related statistics. These weights adjust for the survey design, non-response, and post-stratification to make the results representative of the U.S. population. Weighted means and 95% confidence intervals were utilized to represent variables, ensuring that our estimates accurately reflect the population parameters. In examining variations in baseline traits between participants with and without stroke, continuous variables were analyzed using the student's t-test, which assumes that the data are normally distributed and compares the means of two independent groups. For categorical variables, the chi-square test was employed, assessing the association between two categorical variables by comparing the observed frequencies to the expected frequencies under the null hypothesis of independence. Niacin intake was stratified into four quartiles to evaluate its relationship with stroke, with the lowest quartile (Q1) serving as the reference category. This stratification helps in understanding the dose-response relationship between niacin intake and stroke risk. Assessing the association of niacin with stroke involved employing multivariate logistic regression models. These models were adjusted for potential confounders such as age, sex, BMI, smoking status, physical activity, and other dietary factors. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the strength of association between niacin intake and the likelihood of stroke. To explore the potential non-linear relationship between niacin intake and stroke, restricted cubic spline (RCS) regression with three knots (10th, 50th, and 90th percentiles) was utilized. RCS regression allows for flexibility in modeling non-linear associations by fitting smooth curves to the data without assuming a specific functional form. Subgroup analyses based on age, sex, and BMI were conducted to investigate whether the association between niacin intake and stroke varied across different population subgroups. Interaction terms were included in the regression models to test for statistical interaction, and stratified analyses were performed

to provide subgroup-specific estimates. The statistical analyses were conducted using R software version 4.1.6 (http://www.R-project.org, The R Foundation, Vienna, Austria). All tests were two-tailed, with statistical significance set at a P-value < 0.05, ensuring that our findings are robust and reliable. Sensitivity analyses were also performed to check the stability of our results by using different model specifications and adjusting for additional potential confounders.

Results

Baseline characteristics

We firstly included 101,316 participants from NHANES 1999–2018, after applying the inclusion and exclusion criteria, 39,721 eligible participants were ultimately enrolled (Figure 1). The average age of the entire study cohort was 46.3 years, with almost half (48.6%) being male. A total of 1373 participants (3.5%) were assigned to stroke group. Notably, individuals with stroke history tended to be older (stroke vs. non stroke group: 60.4% vs. 45.9%) and had higher prevalence rates of hypertension (stroke vs. non stroke group: 77.4% vs. 35.7%) and diabetes (stroke vs. non stroke group: 35.5% vs. 11.8%). Detailed demographic and clinical characteristics are provided in Table 1, and Supplementary Table 1 presents a breakdown of these features based on niacin intake quantiles. The mean niacin intake for the overall study population was 21.99 mg, individuals with stroke history showing a lower mean intake of niacin (stroke vs. non stroke group: 21.5 vs. 25.6 mg). The stroke group exhibited elevated levels of HbA1c (stroke vs. non stroke group: 6.04% vs. 5.55%), fasting blood glucose (stroke vs non stroke group: 6.59 mmol/L vs. 5.81 mmol/L), fasting blood insulin (stroke vs. non stroke group: 103.93 pmol/L vs. 76.76 pmol/L), insulin resistance (stroke vs non stroke group: 5.83 vs. 3.57), triglycerides (stroke vs. non stroke group: 1.76 mmol/L vs. 1.49 mmol/L), and hypersensitive C-reactive protein (stroke vs. non stroke group: 0.65 mg/L vs. 0.4 mg/L). Additionally, participants with stroke history demonstrated a decreased level of high-density lipoprotein cholesterol (stroke vs. non stroke group: 1.31 mmol/L vs. 1.37 mmol/L), as outlined in Table 2.

Associations of the intake of niacin with stroke

To investigate the potential association between niacin consumption and stroke, we conducted a thorough multivariate analysis, considering variables like age, gender, ethnicity, education levels, smoking, drinking, hypertension, and diabetes. We found that niacin intake negatively associated with the risk of stroke before (OR: 0.97; 95% CI: 0.96-0.98) and after (OR: 0.98; 95% CI: 0.98-0.99) adjusting covariables. Moreover, participants were evenly divided into quartiles based on niacin intake, revealing that those with higher intake of niacin had the lower stroke risk before and after adjusting covariables (Table 3). Utilizing RCS analysis, we identified a negative nonlinear relationship between niacin intake and stroke risk (P for non-linear trend < 0.05). Before the inflection point (21.8 mg) of the non-linear relationship between niacin intake and the risk of stroke, there is a significant downward trend in the risk of stroke with increasing niacin intake. After the inflection point, the trend of decreasing stroke risk with increasing niacin intake slows down (Figure 2).

Subgroup analysis on the association of the intake of niacin and stroke

We conducted subgroup analyses stratified by gender, age, and BMI to further verify the relationship between niacin intake and the risk of stroke in different populations. The results showed a significant downward trend in the risk of stroke with increasing niacin intake in various groups, including males, females, young, middle-aged, elderly, normal weight, overweight, and obese individuals (Figure 3). This confirms the stability of our findings across different demographic groups. Subgroup analyses of RCS were also conducted across diverse populations, revealing a negative nonlinear relationship between niacin intake and stroke in most groups (Figures 4A–D). Notably, the inflection points for this nonlinear association varied between males and females. Specifically, in females, the inflection point was identified at 19.2 mg, whereas in males, it occurred at 25.8 mg (Figure 4A). It is important to highlight that among overweight participants, the association between dietary niacin intake and stroke was U-shaped. After the inflection point, the stroke risk increased with the increase of niacin intake (Figure 4C).

Sensitive analysis

There are some drawbacks of employing weighted analysis methods in the NHANES analysis. Weighted analysis is often utilized to account for sampling biases and ensure that the findings are reflective of the broader population. However, it's important to consider that the weights are based on certain assumptions, and if these assumptions are not met, the results may be affected. One limitation is the reliance on self-reported data, which introduces the possibility of reporting errors or biases. Additionally, the weights are calculated based on specific demographic characteristics, and any changes or inaccuracies in these characteristics may impact the validity of the weighted analysis. Moreover, the effectiveness of the weighting method depends on the availability and accuracy of the data used for weight calculation. Furthermore, the use of weighted analysis assumes that the sampling design is adequately representative of the entire population. If there are limitations or shortcomings in the sampling approach, it may compromise the generalizability of the results. Therefore, in the present study, we also employed unweighted logistic regression to further confirm the conclusion. We found that the results of unweighted logistic regression were in accordance with main analysis using weighted logistic regression. Niacin intake negatively associated with the risk of stroke before (OR: 0.97; 95% CI: 0.96-0.97) and after (OR: 0.98; 95% CI: 0.97-0.98) adjusting covariables (Table 4).

TABLE 1 Baseline Characteristics Grouped by Stroke.

Variables	Overall (*n* = 39721)	Non- Stroke (*n* = 38375)	Stroke (*n* = 1346)	*P* value
Age, years				< 0.001***
18–40 years	38.94 [37.49, 40.39]	39.72 [38.71, 40.73]	8.46 [6.39, 10.52]	
40–60 years	39.94 [38.17, 41.70]	40.05 [39.22, 40.88]	35.32 [32.15, 38.50]	
> 60 years	21.12 [19.99, 22.26]	20.23 [19.49, 20.97]	56.22 [52.98, 59.45]	
Sex-male, %	48.55 [46.76, 50.35]	48.65 [48.14, 49.16]	44.71 [41.62, 47.79]	0.02*
Race, %				< 0.001***
Non-Hispanic White	69.29 [65.14, 73.44]	69.31 [67.31, 71.31]	68.41 [64.57, 72.24]	
Non-Hispanic Black	11.03 [10.10, 11.96]	10.90 [9.81, 11.99]	16.26 [13.96, 18.56]	
Mexican American	7.84 [6.93, 8.75]	7.91 [6.90, 8.92]	5.14 [3.86, 6.41]	
Other Hispanic	5.56 [4.67, 6.45]	5.61 [4.70, 6.52]	3.64 [2.25, 5.03]	
Other	6.28 [5.79, 6.77]	6.27 [5.75, 6.79]	6.56 [4.66, 8.45]	
Smoking, %	21.54 [20.45, 22.62]	21.36 [20.60, 22.12]	28.78 [25.64, 31.92]	< 0.001***
Drinking, %	83.74 [80.53, 86.96]	89.20 [88.31, 90.09]	84.94 [82.06, 87.81]	< 0.001***
Education level, %				< 0.001***
Below high school	5.08 [4.70, 5.46]	4.96 [4.58, 5.34]	9.97 [7.91, 12.02]	
High school	34.84 [33.07, 36.61]	34.55 [33.33, 35.76]	47.54 [44.02, 51.06]	
Above high school	60.00 [57.45, 62.55]	60.49 [59.13, 61.85]	42.50 [38.84, 46.15]	
SBP, mmHg	121.61 [121.27, 121.94]	121.39 [121.06, 121.73]	130.10 [128.54, 131.67]	< 0.001***
DBP, mmHg	71.66 [71.36, 71.95]	71.68 [71.38, 71.98]	70.70 [69.87, 71.54]	0.02*
DM, %	12.34 [11.72, 12.95]	11.75 [11.27, 12.22]	35.48 [31.99, 38.98]	< 0.001***
eGFR, ml/min/1.73m^2	94.75 [94.26, 95.24]	95.20 [94.71, 95.69]	76.76 [75.04, 78.48]	< 0.001***
RBC, × 10^9/L	4.72 [4.71, 4.74]	4.73 [4.72, 4.74]	4.59 [4.55, 4.64]	< 0.001***
WBC, × 10^9/L	7.24 [7.20, 7.29]	7.23 [7.19, 7.28]	7.51 [7.36, 7.66]	< 0.001***
NE, × 10^9/L	4.29 [4.26, 4.32]	4.28 [4.25, 4.32]	4.57 [4.46, 4.69]	< 0.001***
Monocyte, × 10^9/L	0.56 [0.55, 0.56]	0.56 [0.55, 0.56]	0.59 [0.58, 0.61]	< 0.001***
LY, × 10^9/L	2.14 [2.13, 2.16]	2.15 [2.13, 2.16]	2.07 [2.02, 2.12]	0.01*
PLT, × 10^6/L	255.09 [253.69, 256.49]	255.28 [253.90, 256.66]	247.61 [241.09, 254.13]	0.02*
Hemoglobin, g/L	14.36 [14.32, 14.40]	14.37 [14.33, 14.41]	14.01 [13.88, 14.13]	< 0.001***
CHD, %	3.18 [2.89, 3.47]	2.80 [2.54, 3.05]	18.52 [15.81, 21.23]	< 0.001***
Angina, %	2.24 [2.00, 2.47]	1.97 [1.78, 2.17]	12.89 [10.33, 15.45]	< 0.001***
HF, %	2.01 [1.83, 2.19]	1.67 [1.51, 1.83]	15.73 [13.29, 18.17]	< 0.001***
Hypertension, %	36.69 [35.10, 38.29]	35.66 [34.78, 36.54]	77.36 [74.34, 80.38]	< 0.001***
Heart attack, %	3.17 [2.90, 3.44]	2.73 [2.52, 2.95]	20.54 [17.59, 23.48]	< 0.001***

Continuous variables are presented as the mean [95% CI], category variables are presented as the proportion [95% CI]. CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; DM, diabetes; eGFR, estimated glomerular filtration rate; BMI, body mass index; WC, waist circumference; RBC, red blood cells; WBC, white blood cells; NE, neutrophils; LY, lymphocytes; PLT, platelets; CHD, coronary artery disease; HF, heart failure. *P-value < 0.05, **P-value < 0.01, ***P-value < 0.001.

Discussion

In the present study, we conducted a cross-sectional analysis to explore the association of dietary niacin intake and stroke risk. We found that there was a negative nonlinear relationship between niacin and stroke. Prior to the inflection point (21.8 mg) in the non-linear correlation between niacin consumption and stroke risk, there is a notable decline in the likelihood of stroke as niacin intake increases. Subsequent to this inflection point, the decrease in stroke risk associated with higher niacin intake exhibits a decelerated trend.

Niacin, also known as vitamin B3, is a water-soluble vitamin essential for various physiological functions in the human body (31). Niacin is found in various foods, including meat, fish, nuts, and grains, and it can also be synthesized by the body from the amino acid tryptophan. Adequate niacin intake is important for maintaining overall health and preventing niacin deficiency, which can lead to a condition known as pellagra (32, 33). Additionally, niacin is sometimes used in higher doses for

TABLE 2 Metabolic Indexes of the Study Population Grouped by Niacin Intake.

Variables	Q1 (0–15.81mg)	Q2 (15.81–21.99mg)	Q3 (21.99–29.85mg)	Q4 (29.85-72.63)w	*P* value
HbA1c, %	5.59 (5.56, 5.62)	5.60 (5.57, 5.62)	5.56 (5.54, 5.58)	5.52(5.50,5.54)	< 0.001***
FBG, mmol/L	5.81 (5.76, 5.86)	5.82 (5.75, 5.88)	5.85 (5.79, 5.90)	5.82(5.76,5.88)	< 0.001***
FBI, pmol/L	78.07 (75.07, 81.06)	77.24 (73.90, 80.58)	76.74 (73.36, 80.12)	77.89(74.76,81.01)	< 0.001***
HOMA-IR	3.72 (3.53, 3.90)	3.55 (3.37, 3.73)	3.65 (3.43, 3.87)	3.60(3.43,3.78)	< 0.001***
TG, mmol/L	1.46 (1.42, 1.50)	1.45 (1.40, 1.50)	1.54 (1.49, 1.59)	1.53(1.48,1.59)	< 0.001***
TC, mmol/L	5.18 (5.15, 5.21)	5.10 (5.07, 5.14)	5.09 (5.06, 5.13)	5.01(4.99,5.04)	< 0.001***
HDL-C, mmol/L	1.41 (1.40, 1.43)	1.40 (1.39, 1.41)	1.36 (1.35, 1.38)	1.31(1.30,1.32)	< 0.001***
LDL-C, mmol/L	3.08 (3.04, 3.11)	3.01 (2.97, 3.05)	3.01 (2.98, 3.05)	2.96(2.93,3.00)	< 0.001***
CRP, mg/L	0.48 (0.46, 0.50)	0.44 (0.41, 0.46)	0.39 (0.37, 0.41)	0.33(0.31,0.35)	< 0.001***

Continuous variables are presented as the mean [95% CI], category variables are presented as the proportion [95% CI]. CI, confidence interval; FBG, fasting blood glucose; FBI, fasting blood insulin; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; CRP, C reactive protein; HOMA-IR, Homeostasis model assessment-insulin resistance; LDL-C, low-density lipoprotein cholesterol. ***P-value < 0.001.

TABLE 3 Weighted Logistic Regression Analysis on the Association between Niacin and Stroke.

	Non-adjusted model		Model I		Model II	
	OR [95% CI]	*P* value	OR [95% CI]	*P* value	OR [95% CI]	*P* value
Continuous Niacin	0.97 [0.96, 0.98]	< 0.001***	0.98 [0.97, 0.99]	< 0.001***	0.98 [0.98, 0.99]	< 0.001***
Q1 (0–15.81mg)	Reference	–	Reference	–	Reference	–
Q2 (15.81–21.99mg)	0.78 [0.65, 0.93]	0.01*	0.81 [0.68, 0.97]	0.02*	0.88 [0.75, 1.05]	0.88
Q3 (21.99–29.85mg)	0.56 [0.46, 0.68]	< 0.001***	0.64 [0.52, 0.79]	< 0.001***	0.68 [0.55, 0.86]	< 0.001***
Q4 (29.85–72.63)	0.43 [0.35, 0.52]	< 0.001***	0.59 [0.47, 0.74]	< 0.001***	0.68 [0.53, 0.86]	< 0.001***

Data are presented as OR (95% CI). Model I adjusted for age, sex, and race/ethnicity. Model II adjusted for age, sex, race, education levels, smoking, drinking, hypertension, DM, and energy intake. ***P-value < 0.001, **P-value < 0.01, *P-value < 0.05.

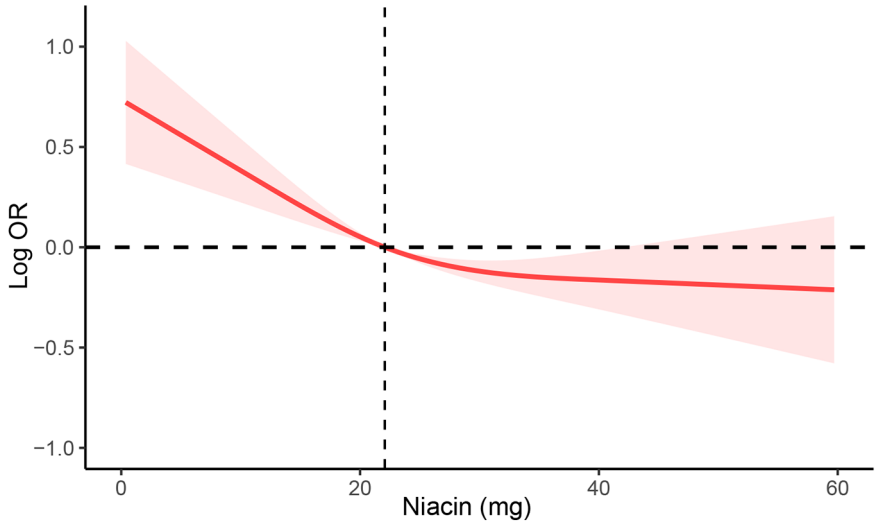

FIGURE 2
RCS analysis of the association between niacin intake and stroke. RCS analysis was adjusted for age, sex, race/ethnicity, education levels, smoking, drinking, hypertension, and DM. RCS, restricted cubic spline; DM, diabetes; OR, odds ratio.

therapeutic purposes, such as managing certain lipid disorders (34, 35). Actually, previous studies have indicated that niacin plays important therapeutic roles in various diseases. Tian et al. revealed a negative relationship between dietary niacin intake and depression risk. Compared to the lowest niacin intake group (Q1, ≤ 15.96 mg/day), the adjusted odds ratios (OR) for depression in the higher intake groups (Q2, Q3, and Q4) showed a decreasing trend, with the lowest risk observed in Q4 (≥ 32.29 mg/day). The relationship remained consistent across different demographic subgroups, including sex, age, and BMI

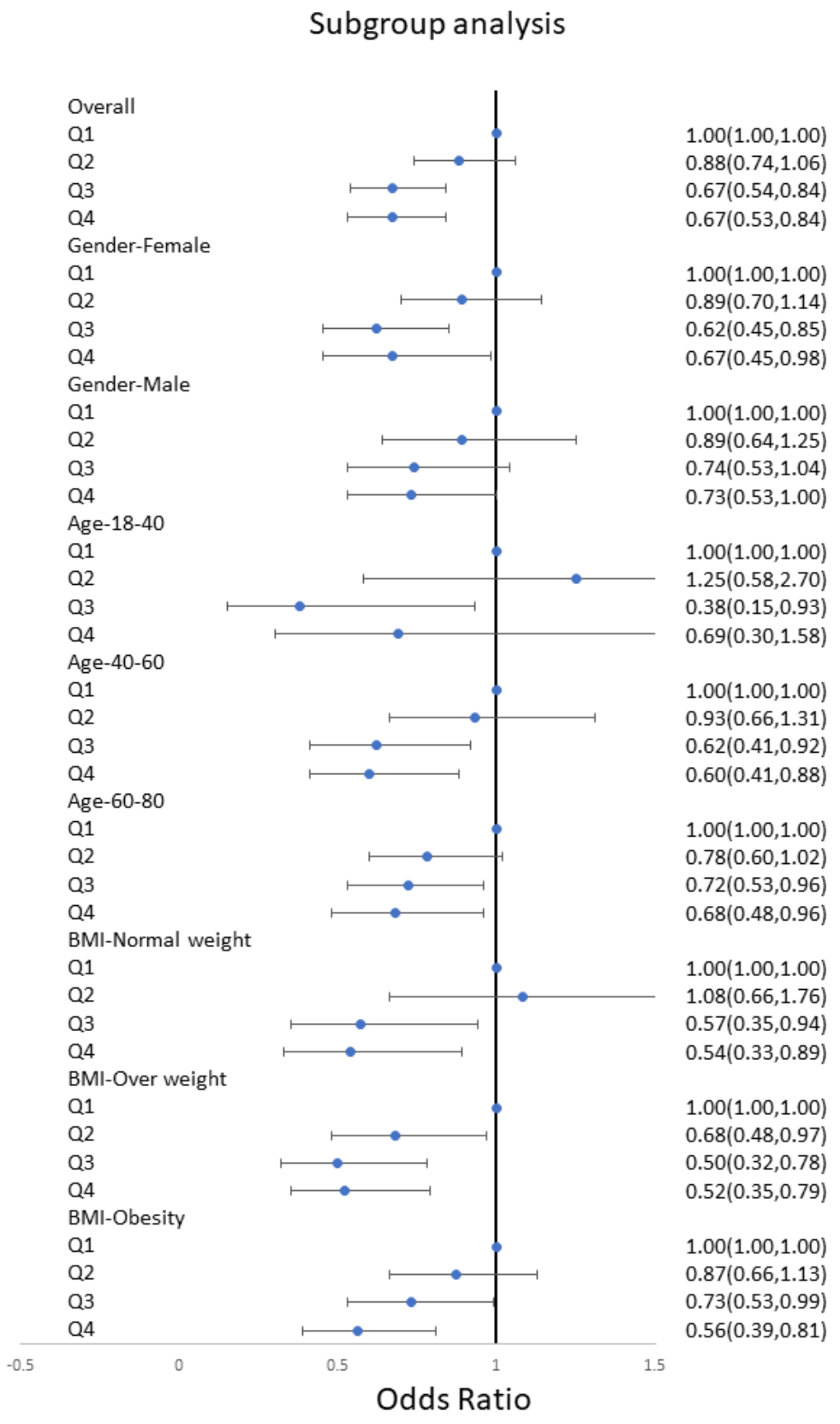

FIGURE 3
Subgroups multivariate logistic regression analyses for the association between niacin and stroke among different populations. Analyses were stratified by age, sex, BMI. Multivariate logistic regression analyses were adjusted for age, sex, race/ethnicity, education levels, smoking, drinking, hypertension, and DM. DM, diabetes; BMI, body mass index; OR, odds ratio.

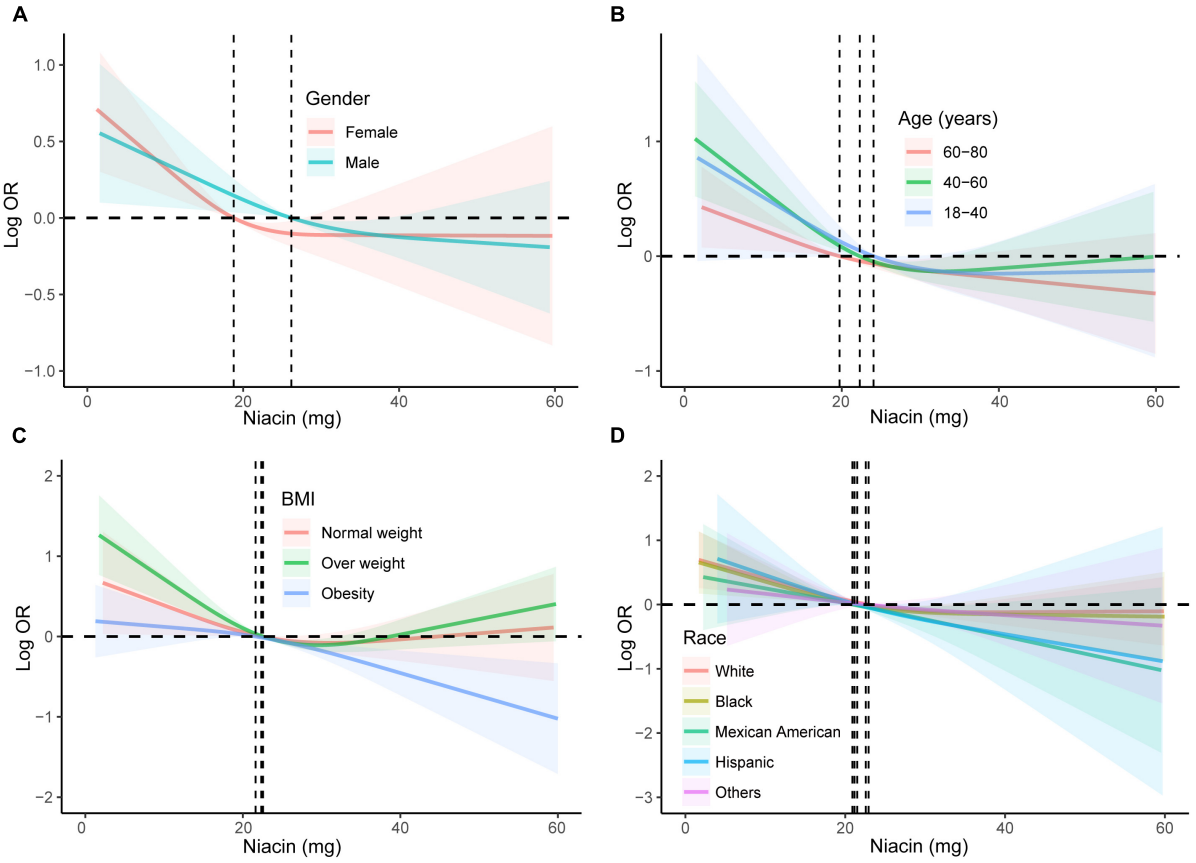

FIGURE 4
Subgroup analyses using RCS were conducted to examine the association between niacin and stroke across diverse demographic groups. The analyses were stratified based on gender **(A)**, age **(B)**, BMI **(C)**, and race/ethnicity **(D)**. Adjustment for potential confounders, including age, gender, race/ethnicity, education levels, smoking, drinking, hypertension, DM, and energy intake, was performed in the RCS analyses. RCS, restricted cubic spline; DM, diabetes; BMI, body mass index; OR, odds ratio.

TABLE 4 **Unweighted Logistic Regression Analysis on the Association between Niacin and Stroke.**

	Non-adjusted model		Model I		Model II	
	OR [95% CI]	*P* value	OR [95% CI]	*P* value	OR [95% CI]	*P* value
Continuous Niacin	0.97 [0.96, 0.97]	< 0.001***	0.98 [0.97, 0.98]	< 0.001***	0.98 [0.98, 0.99]	< 0.001***
Q1 (0–15.81mg)	Reference	–	Reference	–	Reference	-
Q2 (15.81–21.99mg)	0.75 [0.65, 0.86]	0.01*	0.80 [0.70, 0.92]	0.02*	0.86 [0.73, 1.00]	0.07
Q3 (21.99–29.85mg)	0.58 [0.50, 0.68]	< 0.001***	0.68 [0.59, 0.79]	< 0.001***	0.70 [0.6, 0.83]	< 0.001***
Q4 (29.85–72.63)	0.41 [0.35, 0.48]	< 0.001***	0.58 [0.49, 0.69]	< 0.001***	0.63 [0.52, 0.79]	< 0.001***

Data are presented as OR (95% CI). Model I adjusted for age, sex, and race/ethnicity. Model II adjusted for age, sex, race, education levels, smoking, drinking, hypertension, DM, and energy intake. ***P-value < 0.001, **P-value < 0.01, *P-value < 0.05.

(36). Results from Xiang et al. (37) indicated a positive correlation between higher dietary niacin intake and several favorable outcomes. Increased niacin intake was associated with higher grip strength, total lean mass, appendicular lean mass, and total bone mineral content. Conversely, higher niacin intake showed a negative association with total fat, trunk fat, and sarcopenia risk. Notably, dietary niacin supplementation also demonstrated a significant reduction in homeostasis model assessment of insulin resistance (HOMA-IR), fasting blood glucose (in participants without diabetes), and fasting insulin (37). Lee et al. (38) also conducted a cross-section analysis and found that higher levels of niacin intake were associated with decreased odds of glaucoma overall and in women (38). However, to the best of our knowledge, the association of niacin intake and stroke risk remains exclusive.

Risk factors for stroke include high blood pressure, smoking, diabetes, obesity, and a sedentary lifestyle. Age, family history, and certain medical conditions also contribute to the risk (39, 40). Prevention measures often involve lifestyle changes such as maintaining a healthy diet, exercising regularly, managing blood pressure, and avoiding smoking and excessive alcohol consumption (41, 42). Many researchers have studied the risk factors for

stroke in the NHANES database. There is an interesting study focused on the association of urinary paraxanthine levels and stroke risk. Authors found that there was a negative correlation between urinary paraxanthine levels and stroke risk. However, the negative association of urinary caffeine levels with stroke incidence was observed specifically in Mexican Americans, with no evident correlation in other populations, implying potential predictive and diagnostic implications in clinical practice (43). Another study also based on NHANES database also indicated a U-shaped correlation exists between serum uric acid levels and the risk of stroke. Both low and high SUA levels elevate the risk of stroke in distinct populations, with the exception being the other Hispanic population. Effective early management of SUA is crucial for preventing strokes in high-risk populations (44). Zhao et al. (45) utilized a larger sample size drawing on data from the NHANES spanning 2011 to 2018 to explore the association of blood selenium levels and the risk of stroke. With 13,755 adults aged 20 years and above, multivariate logistic regression models and dose-response analyses were employed. In the fully adjusted model, the highest tertile of blood selenium levels was also negatively associated with stroke compared to the lowest tertile (OR = 0.70, 95% CI: 0.53-0.93, P for trend = 0.016) (45). In the present study, we also utilized a large sample size from NHANES database to explore the relationship between niacin intake and stroke risk. We found that increased niacin intake may have protective effect on prevention of stroke. Especially prior of the inflection point of 21.8 mg, the stroke risk decreased significantly with the increase of niacin intake. It is worth noting that the inflection points vary among different populations. For instance, the inflection point for males is 25.8 mg, and for females, it is 19.2 mg. This variability may provide insights for the clinical application of niacin.

Currently, the exact mechanism of Niacin in preventing strokes is not fully understood, but Niacin may prevent the occurrence of strokes through the following mechanisms. Firstly, Niacin has a lipid-regulating effect, especially in lowering low-density lipoprotein cholesterol (LDL-C) levels and increasing high-density lipoprotein cholesterol (HDL-C) levels (21). By reducing lipid deposition on arterial walls, Niacin may help prevent atherosclerosis, thereby reducing the risk of strokes. Niacin possesses antioxidant properties, aiding in neutralizing free radicals and alleviating oxidative stress on blood vessels (46–48). This may contribute to maintaining vascular health and reducing the risk of strokes. Niacin is believed to have anti-inflammatory effects, mitigating inflammatory responses (49–51). As inflammation is associated with atherosclerosis and stroke occurrence, the anti-inflammatory effects of Niacin may contribute to stroke prevention. Finally, Niacin may have a vasodilatory effect by promoting the production of nitric oxide, contributing to the maintenance of vascular elasticity and function, ultimately reducing the risk of strokes (52, 53). However, these protective mechanisms are relatively superficial, and a more in-depth exploration of molecular biological mechanisms requires further investigation through animal experiments. The specific protective effects of niacin also need validation through large-scale prospective clinical trials.

Our study has several limitations that should be acknowledged and considered in future research: (1) The cross-sectional design inherently hinders establishing causality between niacin and stroke (54); (2) Using a single 24-hour recall may not be the optimal method for calculating habitual dietary intake at an individual level due to day-to-day variations. The large number of participants in NHANES surveys may limit the practical application of more accurate options [e.g., multiple 24-hour recalls, food frequency questionnaire (FFQ)] in exploring the long-term link between dietary intake and hypertension. This issue should be addressed in future studies utilizing NHANES dietary information (55); moreover, tryptophan, an essential amino acid, can be converted into niacin through metabolic pathways, thereby influencing overall niacin levels. The absence of data on tryptophan in the study's database prevents a comprehensive analysis of niacin metabolism and its dietary implications. Future research should consider incorporating measures of tryptophan alongside niacin intake to provide a more accurate assessment of their interplay and nutritional impact; (3) Despite attempts to include numerous covariates to control confounding bias, stroke is a complex disorder influenced by multiple genetic, behavioral, and environmental factors. Unidentified confounders may exist, affecting the pathogenesis and progression of stroke, as not all relevant factors are explicitly documented in the NHANES database; Self-reporting is a convenient means of obtaining information about dietary intake and hypertension occurrence among NHANES participants. However, this method may introduce recall bias, and caution is warranted during the analysis and interpretation of the data.

Conclusion

We utilized a substantial sample size from the NHANES database to examine the association between niacin intake and the risk of stroke. Our findings suggest that heightened niacin intake may confer a protective effect in preventing strokes. There is a negative nonlinear association of niacin intake and stroke. Notably, the inflection points exhibit variations across diverse populations. This diversity in inflection points could offer valuable insights for the clinical utilization of niacin.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by The NCHS Ethics Review Board protects the rights and welfare of NHANES participants. The NHANES protocol complies with the U.S. Department of Health and Human Services Policy for the Protection of Human Research Subjects. NCHS IRB/ERC Protocol number: 2011-17. Ethical review and approval were waived for this study as it solely used publicly available data for research and publication. The studies were conducted in accordance with the

local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

J-YQ: Formal analysis, Investigation, Methodology, Resources, Validation, Writing—original draft. W-HZ: Conceptualization, Data curation, Validation, Writing—original draft. X-MZ: Data curation, Formal analysis, Writing—original draft. L-DW: Conceptualization, Supervision, Writing—original draft, Writing—review and editing. J-HH: Conceptualization, Data curation, Writing—review and editing. JZ: Conceptualization, Writing—original draft, Writing—review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1391023/full#supplementary-material

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Is oxidative stress - antioxidants imbalance the physiopathogenic core in pediatric obesity?

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Despite the early recognition of obesity as an epidemic with global implications, research on its pathogenesis and therapeutic approach is still on the rise. The literature of the 21st century records an excess weight found in up to 1/3 of children. Both the determining factors and its systemic effects are multiple and variable. Regarding its involvement in the potentiation of cardio-vascular, pulmonary, digestive, metabolic, neuro-psychic or even dermatological diseases, the information is already broadly outlined. The connection between the underlying disease and the associated comorbidities seems to be partially attributable to oxidative stress. In addition to these, and in the light of the recent COVID-19 pandemic, the role played by oxidative stress in the induction, maintenance and potentiation of chronic inflammation among overweight children and adolescents becomes a topic of interest again. Thus, this review's purpose is to update general data on obesity, with an emphasis on the physiopathological mechanisms that underlie it and involve oxidative stress. At the same time, we briefly present the latest principles of pathology diagnosis and management. Among these, we will mainly emphasize the impact played by endogenous and exogenous antioxidants in the evolutionary course of pediatric obesity. In order to achieve our objectives, we will refer to the most recent studies published in the specialized literature.

KEYWORDS

oxidative stress, endogenous antioxidant systems, exogenous antioxidants, obesity, diet, immunity, child

1 Introduction

Being defined as an increase in the body mass index (BMI) above the 95th percentile/at +1-2 standard deviations compared to the reference average for age and sex, overweight or obesity is a chronic condition with multisystemic effects. Thus, it is frequently associated with hypertension, metabolic disorders (e.g., dyslipidemia, prediabetes or type 2 diabetes), neuro-psychological, gastroenterological (e.g., non-alcoholic fatty liver, gallstones, gastroesophageal reflux), renal, respiratory, orthopedic damage or dermatological (e.g., atopic dermatitis, acanthosis nigricans) (1–6). Also, a positive correlation was also identified with regard to sleep apnea or the increase in the flu severity score among children with obesity (7, 8).

Recent estimates according to the World Obesity Atlas 2023 place pediatric obesity as a chronic condition that affects 2.6 million children under the age of 5 and up to 175 million children between the ages of 5 and 19. The gender ratio in the second category is against girls (103 million cases) (9). Regarding the division by sex, it is certified that girls/women present an increased risk compared to boys partly due to hormonal variability (10). The highest incidence is noted in Western countries, while in low-income countries or in countries with a low development index, pediatric obesity is better represented among families with above-average income (11). An additive risk factor is identified among "medically complex" children, namely those children with chronic health conditions, significant functional limitations and increased risk of hospitalization (12). The prevalence of childhood obesity has grown rapidly in the last 40 years, doubling. According to current trends, it is predicted that in approximately 30 years, up to 25% of children will suffer from excessive weight gain (10, 13). In addition, the literature attests to the direct risk of developing obesity in adult life of the population with excess weight in childhood (14). In this situation, the pediatrician has a key role in reducing the global effects of obesity. Due to the nature of the population, he cares for, knowing and countering the pathological processes that disrupt the homeostasis of the internal environment in obesity and the associated comorbidities will lead, in the long term, to reducing the burden on adults and the medical system (15). To facilitate inclusion in risk groups and reduce childhood obesity, Sonoda R. et al. imagined a prediction model based on seven binary variables (16).

The defining imbalance of obesity is represented by a high caloric intake, doubled by a low consumption. Their main regulators are metabolic rate, appetite, eating habits and lifestyle dynamics. Genetic factors and individual characteristics (e.g., birth weight, breastfeeding period) may predispose to obesity, although a more important imprint is attributed to the environment. Therefore, the increase in the incidence of excess weight in recent years can be more correctly attributed to environmental changes, educational deficiencies or western-type diets. In this direction, current research attests to the involvement of numerous biomarkers (microRNA, adipocyte balance, oxidative stress, blood cell profile, nutrients and microbiota) determinants of the development of excess weight and systemic damage (2, 13, 17–19). Therefore, in addition to the disturbances stated above, excessive weight gain together with the accompanying chronic inflammation represents a continuous source of oxidative stress. The latter seems to be the crossroads in triggering the comorbidities that accompany obesity. Therefore, stimulating interest in research and countering it can be crucial initiatives, additive to new prevention strategies, to improve quality of life and ameliorate systemic decline (20, 21).

Therefore, knowing the global impact of obesity and its comorbidities, both in pediatric and adult age, we tried to broaden the horizons in terms of understanding the physiopathological mechanisms underlying the disturbances and the means by which they can be counteract. In this sense, we chose as a topic of interest the implications of oxidative stress in excessive weight gain. The antioxidant substances are multiple, they can be briefly distinguished into endogenous and exogenous. Although their importance is similar, in the management of the overweight patient, counteracting the main nutritional deficits is essential, both for the restoration of adequate levels, and from the perspective of the function of cofactors for the endogenous substances played by some of them. We caried out a narrative review of the scientific literature from the last decades, by accessing international databases (PubMed, Google Scholar, Web of Science, Scopus and Embase). To facilitate the search, we used terms such as "obesity", "oxidative stress", "antioxidant enzymes", "exogenous antioxidants", "antioxidant vitamins", "antioxidant trace minerals" or "oxidative stress modulators".

2 The impact of oxidative stress in the dynamics of obesity

Obesity has multiple causal determinations. Among these we note the environmental factors (stress, drugs, surgical interventions, chemical substances, diet, sleep schedule, physical activity), genetic predisposition, as well as the disruption of the homeostasis of the internal environment, objectified by oxidative stress reactions. Therefore, maintaining the redox balance in the adipose tissue is an important objective due to its implications in the decrease of the organic antioxidant capacity, the formation of free radicals and reactive species and in the pathogenesis of the metabolic syndrome associated with obesity (22–24).

Oxidative stress is defined as the disturbance of the balance between the production of oxidizing agents and the antioxidant defense, materialized by the formation of free radicals. Biochemical substrates that interfere with its production include the mitochondrial respiratory chain and enzymes (e.g., nicotinamide adenine dinucleotide phosphate oxidase, xanthine oxidase, lipoxygenases, cyclooxygenases, cytochrome P450 enzymes, or uncoupled nitric oxide synthases). This results in reactive species derived from oxygen and nitrogen (superoxide anion radicals, hydroxyl, alkoxyl, lipidic peroxyl, nitric oxide and peroxynitrite). It imprints a state of suffering on the body, sensitizing it. There is an alteration of the intra- and extra-cellular components. It is objectified by structural and functional imbalances of nucleic acids, proteins or lipids. Among its consequences, we note the

over-expression of oncogenic genes, the generation of mutagenic compounds, the promotion of atherogenic activity or inflammation. Current research incriminates the existence of oxidative stress as a physiopathogenic mechanism in a variety of pathologies, depending on genetic susceptibility. Among these we highlight hypertension, atherosclerosis, obstructive pulmonary disease, diabetes, osteoporosis, cancer or even infertility (25–31).

The main method to combat the negative effects of oxidative stress is the detoxification of metabolic byproducts. This is done by enzymatic or non-enzymatic components. The first category includes superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase, glutathione S-transferase, catalase (CAT), thioredoxin reductase, peroxiredoxins (Prx), ubiquinone oxidoreductase and heme oxygenase-1 (HO-1). Recently introduced in research, we find the paraoxonase family (PON) or aryl dialkyl phosphatases, enzymes strongly correlated with the accompanying pathologies of obesity (32). Another important determinant is nuclear factor erythroid 2 (Nrf2), defined as a regulator of cellular resistance to oxidants. Its action is manifested on a wide range of genes with implications in antioxidant modulation, immune or inflammatory responses, tissue remodeling, carcinogenesis and cognitive balance. Knowing and understanding its roles can lead to the explanation of the mechanisms underlying the correlation between oxidative stress and induced pathologies. At the same time, it lays the foundations for new therapeutic perspectives (33, 34). Melatonin is another magical compound, with functions in regulating the circadian rhythm, sleep, inhibiting cancer, but also detoxifying free radicals (35). Dietary antioxidant substances (non-enzymatic) such as vitamin A, C, E and other plant compounds (flavonoids, tannins, lignins) are added to all of these. Likewise, the trace elements (zinc, manganese, selenium) should not be lost sight of, substances assimilated as enzyme regulators (32).

Oxidative stress in obesity has been shown to be directly correlated with fat mass. Precipitating factors in this case include altered nutritional balance, hyperglycemia, hyperlipidemia and chronic inflammation. Also, the high-carbohydrate diet potentiates oxidative stress, an interaction objectified by increasing lipid peroxidation and protein carbonylation, doubled by the reduction of glutathione and antioxidant levels. Other classical risk factors incriminated are pollution, radiation, pesticides or other toxic substances, infections and surgical interventions. The consequences are diverse, among which we mention the influence of myocardial contractility, vascular remodeling, insulin resistance or adiponectin secretion. This explains the correlation between obesity, inflammation and various chronic pathologies that make up the metabolic syndrome (diabetes, heart disease) or not (carcinogenesis, asthma, COVID-19) (36–39). Summarizing the above, Figure 1 shows the enzymatic and non-enzymatic balance involved in the pathogenesis of oxidative stress in obesity. Imbalances at this level interfere, in addition to inflammation, with mitochondrial activity, adipogenesis, lipolysis and lipogenesis, appetite and iron metabolism.

2.1 Mitochondrial activity

The main roles of mitochondria are represented by production of energy (adenosine triphosphate – ATP) depending on cellular needs and fighting infections by promoting reactive oxygen species (40). In this sense, mitochondrial/peroxisomal oxidation of fatty acids, as well as excessive oxygen consumption can produce reactive oxygen species (41). Mainly, during the conversion to ATP, a small amount of high-energy electrons may deviate from the programmed path, thus generating superoxide radicals. They are enzymatically or spontaneously transformed into hydrogen peroxide and later into hydroxyl radicals, dangerous molecules (they can indicate cell apoptosis). The effective antioxidant mechanism in this sense is superoxide dismutase. The enzyme switches the process towards the formation of hydrogen peroxide. Later glutathione peroxidase will determine its transformation into

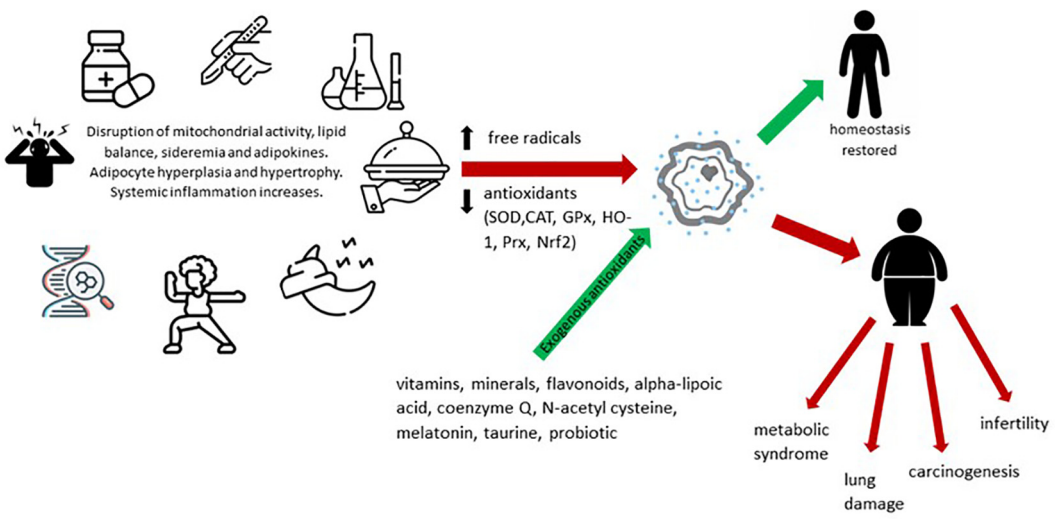

FIGURE 1
Dynamics of oxidative stress and its implications in pathogenesis of obesity.

water (42). Inflammation together with the overproduction of free radicals in obesity predispose to the appearance and maintenance of mitochondrial dysfunction. This is defined as decreased biogenesis, altered membrane potential, altered mitochondrial gene expression, and reduced ATP production (43). Another variable encountered in this case is the excess supply of nutrients. The consequence is the overwhelm of the Krebs cycle and the mitochondrial respiratory chain. This is how mitochondrial dysfunction is precipitated, which results in the formation of an increased amount of reactive oxygen species. The two mechanisms therefore intertwine, aggravating in parallel both mitochondrial dysfunction, as well as inflammation and increased insulin resistance specific to obesity (40, 42). The exposed changes, related to mitochondrial biogenesis, enzyme balance and the consequences of the two, were also reported by Zamora-Mendoza R. et al. (44). Summarizing the above, Figure 2 illustrates the way in which mitochondrial dysfunction induces an increase in oxidative status and disruption of the homeostasis of the internal environment.

2.2 Adipogenesis

Adipose tissue normally represents up to 30% of body mass. Its roles in the body are variable, being briefly divided into structural (support) and functional. Depending on its distribution, it can be classified into subcutaneous adipose tissue and visceral adipose tissue. Its structural and functional characteristics facilitate its division into 3 categories: white, brown or beige adipose tissue. Functionally, the main role of white adipocytes is to store energy, while brown adipocytes intervene in thermogenesis. Thermogenesis occurs by stimulating uncoupling proteins, the result being the conversion to produce heat instead of ATP. Beige adipocytes share similar characteristics to brown ones, but being distributed in white adipose tissue deposits (45). Brown adipose tissue seems to have a metabolic activity directly correlated with the volume of muscle mass; an aspect partially explained by the presence of common precursors with muscle cells (myf5-positive precursors similar to myoblasts). Also, it is better represented in the pediatric population, unlike adults. The investigation recommended for its evaluation is positron emission tomography, although the results may be influenced by age, degree of sexual development, medication used, fat accumulation, disease state, plasma glucose concentration, radiotracer dose, season or temperature during examinations. Antioxidant supplementation has beneficial effects on the dynamics of brown adipose tissue (45–47). Current data from the literature attests to the discrepancy between white adipose tissue/brown adipose tissue in the pathogenesis of obesity. If the excessive distribution of the first one is found in the case of overweight people, the brown tissue protects against weight gain and its comorbidities (insulin resistance, dyslipidemia). However, the way of distribution of white adipose tissue should not be neglected. It differentiates pathological obesity from "metabolically healthy" obesity (Figure 3). The second case is characterized by the expansion of subcutaneous deposits, adipocyte hyperplasia and limited ectopic lipid deposition (48, 49).

The imbalance in adipose tissue, characteristic of obesity (hypertrophy, hyperplasia), is accompanied by a pro-inflammatory state, mitochondrial and endoplasmic reticulum dysfunction and, consequently, increased oxidative stress at this level. This in turn induces disturbances in the production of adipokines. The link between the two is strengthened by the direct correlation between oxidative damage markers and BMI, body fat percentage, low-density lipoprotein (LDL) oxidation, and triglyceride (TG) levels. The consequences are reflected in the increased risk of developing comorbidities in the medium and long term (50, 51). In agreement with the functional characteristics of adipose tissue, studies on adults attest to lower levels of oxidative stress in men, compared to women. At the same time, the correlation with environmental factors is certified (e.g., the use of oral contraceptives, hormonal therapies) (52). In turn, oxidative stress promotes inflammation by activating the components of innate immunity and modulating the activation of

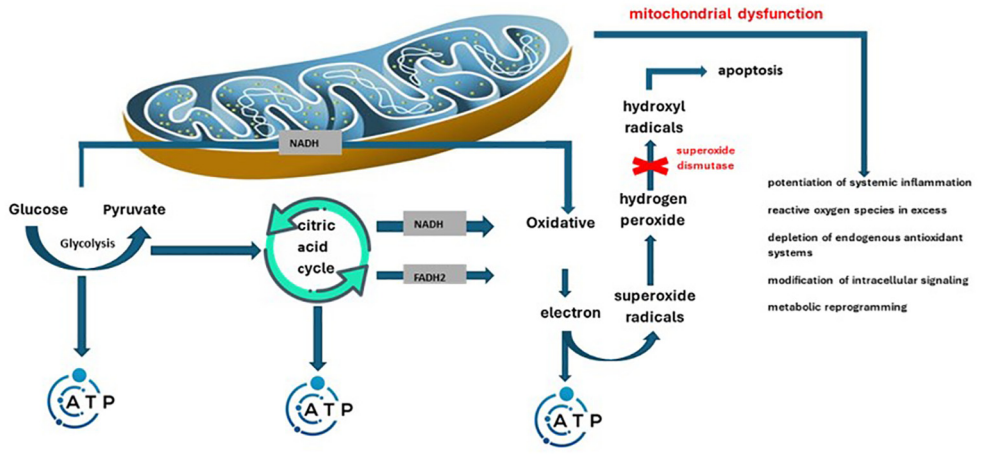

FIGURE 2
Pathological mechanisms through which mitochondrial dysfunction intervenes in the potentiation of the oxidative status and systemic damage.

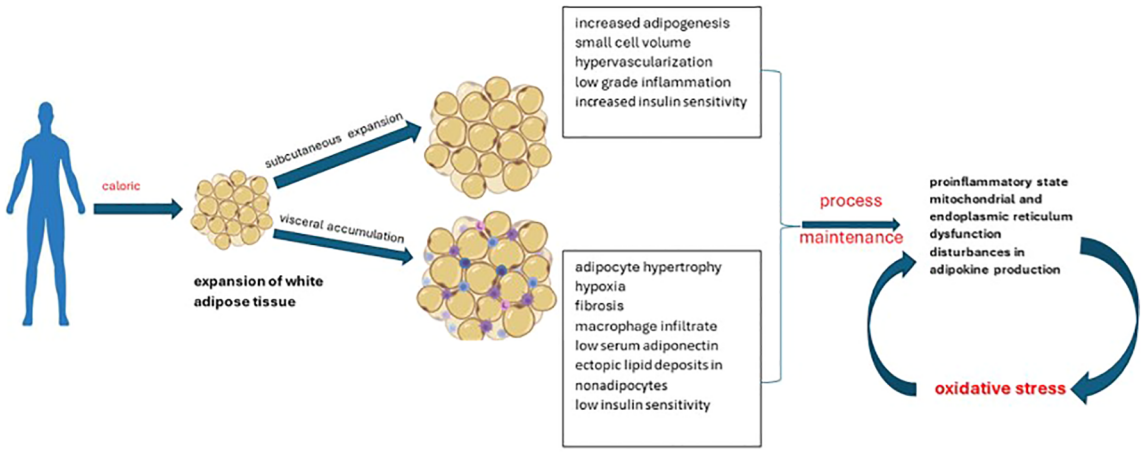

FIGURE 3
Patterns of obesity depending on the expansion of white adipose tissue.

the nucleotide oligomerization domain protein-1 (NOD1). Following the activation of NOD1, there is an increase in NADPH oxidase (NOX) 1 and 4. From a therapeutic point of view, it seems that the manipulation in the descending direction of NOX1/4 decreases oxygen free radicals, in parallel with the increase in catalase-type antioxidant enzymes and SOD (53, 54).

2.3 Lipogenesis and lipolysis

Lipids fulfill important biological roles such as energy source, structural components or signaling mediators. The tissues involved in lipogenesis are the liver and adipose tissue (white/brown). This modulates, in addition to systemic energy homeostasis, the function of the immune and nervous systems. Thus, lipid metabolism disturbances can be accompanied by pathological consequences, partly due to membrane remodeling (55–57). The metabolic integrity of the body can be estimated by evaluating the ability of adipocytes to convert glucose into lipids (*de novo* lipogenesis), a process affected in the case of insulin resistance (58). Therefore, in obesity, the classic lipolytic pathway is switched to an alternative, inflammatory pathway. Reactive oxygen and nitrogen species can influence these pathways at different levels, with consequences dependent on concentration, reactivity and source (Figure 4). Mainly excessive and prolonged lipolysis (lipotoxicity) causes structural and functional changes in adipose tissue. The physiopathogenic cascade ends with the impairment of insulin sensitivity, maintaining the vicious circle of lipolysis. In conclusion, we emphasize the importance of antioxidants in restoring the homeostasis of the internal environment (59, 60).

2.4 Leptin – adiponectin balance

Adipose tissue also fulfills an endocrine role, secreting a series of bioactive molecules (adipokines) that influence the internal balance. Their production is influenced by insulin, catecholamines and adiposity. By this we mark another stage of the physio-

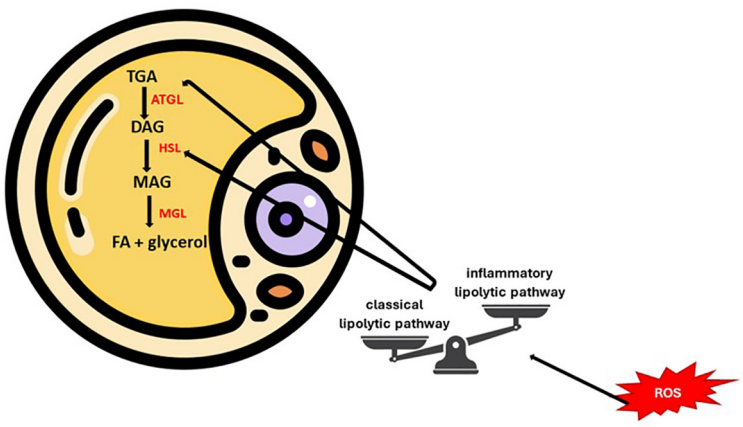

FIGURE 4
Key points of lipolytic balance modulated by oxidative status.

pathological process influenced by oxidative stress, namely the leptin/adiponectin balance. The functions performed by the two molecules are contradictory (Figure 5). Leptin is involved in appetite regulation by modulating dopamine secretion by the limbic system. At the same time, it stimulates oxidative stress, inflammation, thrombogenic risk, arterial stiffness, angiogenesis, atherogenesis and lipolysis, inhibiting lipogenesis. This explains the increased cardiovascular risk characteristic of obesity. At the opposite pole, adiponectin is an anti-inflammatory molecule that increases sensitivity to insulin, reduces the development of atherosclerosis, cell apoptosis and the flow of free fatty acids, in parallel with the increase in their oxidation. Its action is mainly achieved through the interaction with ceramidase, the enzyme involved in the intracellular balance of ceramide. Oxidative stress suppresses its production, thus increasing the risk of associated comorbidities. Supplementation with antioxidants restores the circulatory balance and reduces morbidity. Other adipokines involved in the modulation of comorbidities are adipsin, resistin, visfatin, omentin and apelin (41, 61–64).

2.5 Iron metabolism

Iron metabolism has a double value in obesity, disturbances at this level can be both a consequence of overweight and a cause. The common point between the two entities seems to be represented by macrophages. On the one hand, it can be influenced by chronic inflammation, its deficit affecting the function of hemoproteins and non-heme proteins. In this case, iron deficiency is accompanied by a low value of ferritin, in contrast to that of hepcidin, and a negative correlation between transferrin saturation and adiposity. On the other hand, excess iron can induce the formation of oxygen free radicals (hydroxyl radical) through the Fenton reaction whose course and consequences are illustrated in Figure 6 (65, 66). Added to these are the increase in lipogenesis, the reduction of lipolysis and the promotion of mitochondrial dysfunction (67). This is how ferroptosis (regulated non-apoptotic cell death) occurs, characterized by lipid peroxidation when the endogenous antioxidant status of the cell is compromised. The remedies in this situation are represented by dietary restriction, the use of iron chelators, lipophilic antioxidants (vitamin E), ferostatin-1, liproxstatin-1 or polyphenols (68, 69).

2.6 Inflammation

Chronic, low-grade inflammation is the main mechanism incriminated in promoting obesity-induced damage. This is characterized by a continuous activation of the innate immune system (70). The cause is the accumulation of lipids in adipocytes, leading to adipocyte stress and local hypoxia following tissue hypertrophy and hyperplasia. Thus, necrosis and infiltration by macrophages occurs. There is an increase in the production of pro-inflammatory mediators (interleukin-6 and 1, tumor necrosis factor α, monocyte chemoattractant protein 1) and prothrombotic (plasminogen activator inhibitor-1). Additionally, deficiencies of the oxidizing and antioxidant system are noted. At the same time, the local inflammation spreads to the systemic level (42, 71, 72). Tumor necrosis factor α (TNF-α) has also been linked to endothelial dysfunction, increased atherogenic risk, development of insulin resistance and diabetes. This is achieved through four essential means, namely the activation of nuclear factor κB (NF-κB), increasing the release of free fatty acids in adipocytes, blocking the synthesis of adiponectin and modulating the phosphorylation of tyrosine residues, an essential substrate in intracellular signaling. Regarding IL-6, its levels are directly correlated with BMI, insulin resistance and carbohydrate intolerance. Besides these, it regulates the levels of adipokines (inhibits visfatin and adiponectin) (41).

In conclusion, we present the results obtained by Matusik P. et al. who reiterate, based on their research, the positive correlation between oxidative disturbances, sports, excess adipose tissue and its hormonal activity. However, he notes that in the case of patients practicing sports training (chronic physical activity), the antioxidant defense was more potent. In consensus, Huang CJ.

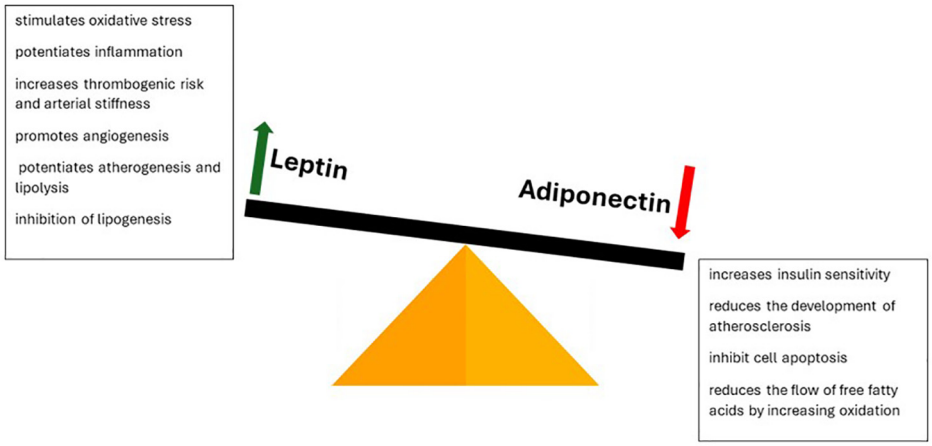

FIGURE 5
Leptin-adiponetic antithesis in dictating obesogenic risk and its complications.

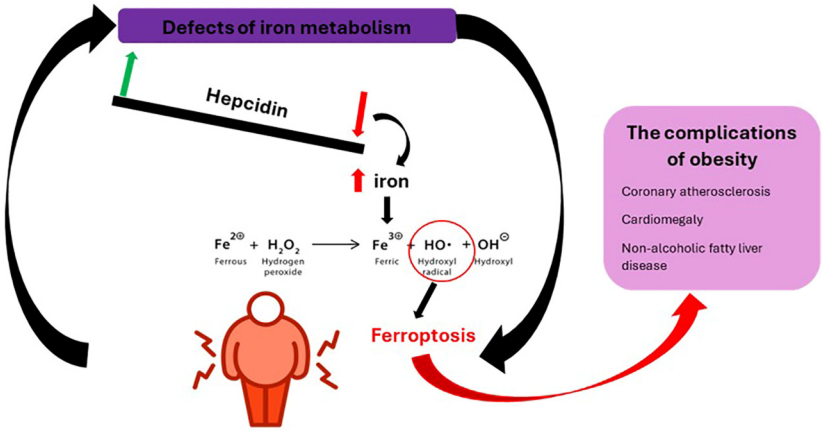

FIGURE 6
The physiopathogenic mechanism of obesity complications following iron metabolic defects.

et al. explains the connection between sport and the oxidant-antioxidant balance, emphasizing the dependence on the individual characteristics of the patient, the type of physical activity practiced, intensity and period. Also, a brief distinction is made of the mechanisms by which aerobic and anaerobic physical activity influence the internal balance. The final result is, in both situations, represented by the increase of stress markers (73, 74). Similar findings have been identified in the literature regarding non-alcoholic fatty liver disease, another component of the metabolic syndrome, frequently associated with obesity (75).

3 Antioxidant systems

Reactive oxygen species in excess can induce apoptosis, necrosis or autophagy. The main pathways targeted in this process are mTOR activity, adenosine monophosphate-activated protein kinase (AMPK), C-Jun-N-terminal kinase (JNK)/P53, and the balance between serine/threonine receptor-interacting protein kinase 3 (RIP3) and RIP1 (76). For these reasons, knowing and potentiating the main endogenous antioxidants is vital in the evolution of patients.

3.1 Enzymatic antioxidants

Superoxide dismutase and catalase are two key enzymes in returning the body to its equilibrium state. The two participate in the elimination of toxic radicals through the initial conversion to hydrogen peroxide (H_2O_2) by SOD, which will then be degraded by catalase into oxygen and water. Thus, to estimate the level of expression of the two in the peripheral sagin cells, Mohseni R. et al. they turned to real-time polymerase chain reaction (PCR). The results confirmed the low levels among obese children, inversely correlated with BMI, fasting blood glucose, insulin resistance, LDL-Cholesterol, TG and systolic blood pressure (77). SOD is divided into 3 isoforms in mammals. These are the cytosolic copper-zinc dimeric form (SOD1), mitochondrial manganese tetrameric SOD (SOD2), and extracellular Cu/Zn tetrameric SOD (SOD3). Among them Erdeve O. et al. demonstrates the involvement of SOD3 in the antioxidant response since childhood (78, 79). At the same time, Özgen İT. et al. underlines the role of genetic polymorphisms (respectively VV alleles) of SOD2 Ala16Val in dictating the risk of developing oxidative stress associated with increased HOMA-IR score (80). In turn, CAT is a peroxisomal antioxidant enzyme, crucial in adipogenesis. Studies on erythrocytes report the possible correlation of single nucleotide polymorphisms (promoter variant -844A/G) and post-translational modifications of CAT with the risk of developing obesity and its comorbidities. At the same time, the low levels of CAT observed in obesity are partially due to increased S-nitrosation of the enzyme (81–83). Paradoxically, it remains contradictory if CAT overexpression in adipose tissue offers any benefit in terms of improving the metabolic profile or the balance of adipogenesis (84).

The main disadvantage of antioxidant enzymes is the inability of exogenous variants to provide benefits to damaged tissues. The main cause is the low permeability of the membrane. Thus, in order to achieve results, recombinant formulas are used, made up of SOD and cell-penetrating proteins (SOD-CPP). This formula can even cross the blood-brain barrier, reducing the expression of inflammatory factors and inhibiting NF-κB signaling pathways (85). Another form used is the SOD mimetic manganese metalloporphyrin (86).

Glutathione peroxidase is an antioxidant enzyme found at the cytoplasmic and mitochondrial level in mammals. The entire superfamily brings together eight isoforms, of which types 1-4 and 6 are selenoproteins, the rest based on cysteine. In humans, the most common isoform involved in oxidative balance is GPx1 (87). As a means of action, it maintains the balance between the necessary/damaging level of cellular oxidants by reducing hydrogen peroxide and soluble lipid hydroperoxides. The source of reducing equivalents used is glutathione. Enzyme balance is important because low levels of hydrogen peroxide are required for physiological processes such as growth factor-mediated signaling,

formation of protein-disulfide bonds, and regulation of normal mitochondrial function. At the same time, a high level of GPx1 can induce dysregulation of islet insulin production and secretion, culminating in diabetes-like phenotypes. The repercussions at the pancreatic level consist of depletion of murine regenerating islet-derived protein 2 (REG2). Thus, maintaining optimal levels of GPx1 is crucial in modulating systemic inflammation and preventing associated metabolic, neurological, cardio-vascular and oncological risks (88–91). In this process, an important role is attributed to glutathione reductase. The enzyme is the main supplier of reduced glutathione, a necessary substrate for the proper development of the redox process (92). Independent of other variables that can influence the redox balance, it seems that seasonal factors (e.g., vitamin D) can modulate the reactions based on glutathione (93). In this sense, vitamin D supplementation showed benefits in increasing glutathione, GPx1 and SOD, reducing oxidative stress and suppressing ferroptosis (by activating the Nrf2 signaling pathway). In parallel, the neuroprotective effect exerted following the hypoxic-ischemic stimulus is noted (94).

Peroxiredoxins represent an enzyme superfamily, dependent on cysteine. Prx reduce over 90% of cellular peroxides. These have six subfamilies in their composition (95). They demonstrated their role in regulating adipogenesis and the metabolic/inflammatory implications of obesity. Among these we note diabetes, atherosclerosis, liver, cardiac or even reproductive damage (spermatogenesis) (96–100). Similar implications are noted in the case of paraoxodaxa-1, the thioredoxin/thioredoxin reductase system (Trx/TrxR), adipokines or heme oxygenase-1 (101–105).

3.2 Non-enzymatic antioxidants

Among the non-enzymatic molecules involved in the clearance of reactive oxygen species and the reduction of their harmful effects, we find vitamins (A, E and C) and other plant compounds (flavonoids, tannins, lignins, carotene, alpha-lipoic acid), chemicals such as coenzyme Q/ubiquinone (MitoQ), N-acetylcysteine (NAC) or trace elements (zinc, manganese, selenium). Compounds with special properties such as melatonin and taurine are added to these (32, 43, 76).

3.2.1 Vitamins

Fat-soluble vitamins (A and E) are dietary constituents whose intestinal passage (absorption/elimination) occurs passively, without energy consumption. Vitamin A can be found as two forms, preformed (all-trans-retinol and its esters) and provitamin A (β-carotene). Of these, β-carotene, similar to other dietary carotenoid products, acts as an antioxidant substrate (106). Similarly, the distribution of vitamin E brings together 8 isoforms (four tocopherols and four tocotrienols - α -, β-, γ-). Vitamin E is ubiquitous in the body's sites, although it lacks the possibility of endogenous synthesis. The functions performed by it spread over the allergic, inflammatory, atherogenic, metabolic (glycemic, lipidic), oncological balance, being also an important pillar in cardioprotection and neuroprotection. It is believed that the antioxidant activity of vitamin E is dependent on the number of methyl groups on the chroman ring. The antioxidant power includes α, β, γ and δ isoforms in descending order, while tocopherol is stronger than tocotrienol (107).

Based on previous findings, Guerendiain M. et al. certifies the involvement of the correlation between optimal levels of fat-soluble vitamins and reduced adiposity, greater weight loss and improved cardio-metabolic profile (108). In addition, Gama Oliveira MN. et al. include in the discussion another predisposing variable, namely the Q223R leptin receptor polymorphism (109). Another concern brought into discussion by Gajewska J. et al. is the risk of hypovitaminosis E or A in obese children, dependent on the BMI curve for weight loss through lifestyle interventions (110).

Vitamin C (ascorbic acid) fulfills in the body both a role in the antioxidant and immune balance (innate or adaptive) and as an enzyme cofactor. Through its functions, it promotes the integrity of the skin barrier, increases chemotaxis, phagocytosis, the generation of reactive oxygen species and microbial killing, reduces necrosis and post-infectious tissue damage and promotes the differentiation and proliferation of T and B lymphocytes. Thus, in inflammations and infections, subject to important reactive oxygen species, vitamin C has a double valence. While its deficiency induces the disruption of immune defense mechanisms, the infections themselves promote the deficiency due to inflammation and nutritional requirements, thus maintaining the vicious circle (111, 112). Current literature attests to the benefit of ascorbic acid in oncology, cardiovascular (hypertension, stroke, atherosclerosis) and nutritional (obesity) conditions. However, we emphasize the existence of gender influence, marked by a negative correlation between vitamin C intake and abdominal obesity in women. The mechanisms probably involved in this are represented by the modulation of adipocyte lipolysis, adrenal glucocorticoid levels, glucose metabolism (improves blood sugar and decreases glycosylation) and leptin secretion and reduces the inflammatory response (113–115). It is known that the optimal intake for a person of approximately 60 kg is 110 mg/day. Carr AC. et al. note that, to avoid vitamin C deficiencies, it is necessary to supplement with 10 mg/day for every 10 kg of excess weight (116).

Comparing the three vitamins, Xie D. et al. notes an increased antioxidant power of vitamin A compared to vitamin C. At the same time, vitamin E is more potent than vitamin A, the combination of the two not registering additional antioxidant benefits (117).

Vitamin D (25-hydroxyvitamin D) is another fat-soluble vitamin with a strong role in homeostasis of the internal environment. The main functions are based on the inflammatory, oxidative and mitochondrial balance. Its balance is influenced by a multitude of physical and environmental factors. In obesity in particular, hypovitaminosis D seems to be due to insufficient food intake, reduced exposure to sunlight due to the inclination towards a sedentary lifestyle, decreased intestinal absorption, hydroxylation in adipose tissue or accumulation in fat. To these is added the genetic predisposition marked by the variation of the vitamin D receptor gene (118–120). Studies on murine models attest to the beneficial effects of vitamin D supplementation on oxidative stress and inflammatory markers in the overweight group (121). Similar

findings were reported by Ionica M. et al., in the study on the adult, overweight and obese population. They also record the inverse correlation between the serum level of the vitamin and the amplitude of adipose oxidative stress (122). Continuing the reasoning, Usman M. et al. objectifies, by studying obese pediatric patients aged between 10-18 years, the positive link between adipose markers and oxidative DNA damage. In conclusion, the three entities (obesity, hypovitaminosis D and DNA damage) represent predictive markers of genomic instabilities (123). In conclusion, Filgueiras MS. et al. underlines the importance of screening for vitamin D status in light of the association with non-conventional cardiometabolic markers (C-reactive protein, IL-6, cathepsin S, vascular cell adhesion molecule-1, malondialdehyde, myeloperoxidase, 3-nitrotyrosine and SOD) in the pediatric population (124). It is estimated that increasing vitamin D by approximately 9 ng/ml would counterbalance this negative effect of visceral adiposity (125).

3.2.2 Microelements

Microelements are important chemical structures in the balance of the internal environment. By their nature, they fulfill multiple functions, entering into the composition of enzymes, vitamins, hormones and pigments. By far the most important role is that of an enzymatic cofactor, modulating organic biochemical processes through their level. Currently, the medical literature attests to the presence of deficiency in trace elements in children and adolescents with obesity. Among these, the biggest shortage seems to be recorded in what concerns copper (126). Although the relationship between obesity and nutritional deficiencies remains open to research, Błoniarz J. et al. links the metabolism of carbohydrates, fats and implicitly the appearance of secondary obesity to the balance of chromium, zinc, copper, manganese, iron or nickel (127). In addition, Luque-Díaz MJ. et al. underlines the existence of hyperzincuria, hypocupruria, hypozincemia and hypercupremia in groups of obese patients, unlike controls (128). Not being the object of the current study, we choose to expose in what follows its implications from the perspective of oxidative stress.

We therefore consider zinc, manganese, selenium and magnesium as the main elements involved in antioxidant regulation. The function of zinc includes a wide range of biological processes such as cell proliferation, immune function and defense against free radicals (through the synthesis of metallothioneins - they reduce hydroxyl radicals and sequester reactive oxygen species), cellular response to oxidative stress, DNA repair, cycle regulation cellular, membrane stabilization, inhibition of the enzyme nicotinamide adenine dinucleotide phosphate oxidase (NADPH-oxidase) and apoptosis. The zinc deficiency, doubled by the high-fat diet, is blamed for the occurrence of cardiac hypertrophy related to obesity. Regarding the role of enzyme cofactor, it influences various structures such as SOD or zinc-α2-glycoprotein (ZAG). The latter is an adipokine with an anti-inflammatory role and in the mobilization of lipids found in reduced quantities in obese patients (129–133). The level of zinc must therefore be kept within normal limits, its marginal deficit may increase the risk of obesity (134). At the same time, Mendes Garrido Abregú F. et al. discuss the importance of adequate zinc levels during breastfeeding. They emphasize that adequate post-weaning zinc diet has a reduced role in preventing cardio-metabolic changes induced by perinatal restriction (135).

Selenium supplementation had, similar to zinc, a beneficial effect in regulating body weight, metabolic and oxidative profile (136, 137). In addition to its antioxidant (main cofactor of GPx) and anti-inflammatory implications, selenium intervenes beneficially in non-alcoholic fatty liver disease induced by obesity. It promotes the synthesis of selenoprotein P1 (SEPP1) which further regulates the Kelch-like ECH-associated protein 1 (KEAP1)/Nrf2 pathway (138, 139). The advantages of exposure to selenium were also documented by Abo El-Magd NF. et al. (140). Last but not least, manganese influences the body's antioxidant balance. It is a redox-active component with a key role in cellular adaptation to oxidative stress. The main functions are cofactor for SOD and substrate for the formation of manganese non-proteinaceous antioxidants. In these processes, the "adversary" is the iron (141, 142).

3.2.3 Other compounds with an antioxidant role

Flavonoids are plant compounds found in fruits and vegetables, known for their strong antioxidant properties. They are estimated to have a diversity that includes six subgroups made up of up to 5000 substances with a common characteristic (skeletal structures with 15 carbon atoms, two phenyl rings and a heterocyclic ring). The most abundant flavonols are quercetin, catechins and kaempferol. They can be used in the prevention or management of obesity and associated diabetes. It exerts its functions on peripheral tissues and pancreatic beta cells, improving insulin secretion and sensitivity. It also regulates inflammation by acting on NF-κB through mitogen-activated protein kinase (MAPK) pathways. Quercetin can inhibit macrophage inflammatory response by activating AMPK phosphorylation and sirtuin 1 (SIRT1) expression (143–145). Besides these, flavonoids modulate thermogenesis, lipogenesis (decrease), lipolysis (increase), energy consumption, food and nutritional intake, B-oxidation of fatty acids and carbohydrate balance. In the oxidizing balance, flavonoids can clean reactive species of oxygen or nitrogen by direct or indirect mechanism (146–148). A special role is attributed to the ability to stabilize the intestinal microbial ecosystem. Thus, we previously demonstrated that intestinal dysbiosis is a process that can be the basis of a wide range of diseases starting from obesity, the associated comorbidities and culminating in gastrointestinal, cardiovascular, renal, atopy or autoimmunity pathologies. The link between them is attributed to the intestines-vital organs axes, intensively studied at the present time (149–156). Finally, Gentile D. et al. underlines the importance of a diet rich in flavonoids in modulating the systemic inflammatory and oxidative balance, with the aim of avoiding obesity and its associated comorbidities (157).

Alpha-lipoic acid (ALA) is another vegetable component with antioxidant effects. Although it is still under study, research on adults attests that supplementation with 600 mg intravenously for 2 weeks improves insulin sensitivity and reduces mitochondrial functional manifestations. Also, the levels of free fatty acids,

dyslipidemia and oxidative/inflammatory markers were reduced, in parallel with adiponectin, which registered an increase. To these is added the positive impact in the recycling of vitamins C and E and the chelation of toxic metals (158–161). Research on murine models is more advanced, indicating similar results. Looking specifically at inflammation and oxidative stress, ALA caused reductions in prostaglandin E2, leukotrienes B4 and C4, T cell proliferation and IL-2 production, and levels of lipid peroxidation products, doubled by increased SOD2 and reduced intracellular glutathione (161–164).

Coenzyme Q10 (ubiquinol/ubiquinone) is a key component in the mitochondrial electron transport chain. One of the causes of ubiquinone deficiency is attributed to the statin-induced lipid-lowering effect by inhibiting the conversion of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA) to mevalonate. The main antioxidant mechanisms attributed to coenzyme Q10 are its action as a cofactor and activator of mitochondrial uncoupling proteins, the ability to accept and donate electrons, the inhibition of lipid and protein peroxidation, the prevention of LDL oxidation and the improvement of the availability of other antioxidants (vitamin C, E, beta-carotene). Thus, a diet with foods rich in coenzyme Q10 (100-150 mg/day) or its supplementation has proven effective in regulating carbohydrate metabolism, improving inflammation and oxidative stress characteristic of obesity/metabolic syndrome (165–168).

The benefits of N-acetyl cysteine (NAC) are multiple and beyond doubt. NAC acts against the complications induced by obesity both by reducing the abnormal pro-inflammatory response and limiting oxidative damage, as well as by inhibiting lipid accumulation by targeting adipogenic transcription factors. Studies on murine models have also demonstrated an effective effect on hyperglycemia, dyslipidemia and oxidative stress (increase the expression of endogenous antioxidant enzymes) occurring consecutively to a diet rich in sucrose (169–172).

The roles of melatonin are vast and still incompletely elucidated. Besides the well-known implications of melatonin in dictating the circadian rhythm, the balance of the intestinal microbiota, sleep disorders and the opioidergic system, it has recently come to the attention of research as a regulatory factor (modulating the activity of melatonin 1 and 2 membrane receptors) of the lipid and tension profile, of glucose metabolism, oxidative stress, inflammation and adipose tissue. Thus, melatonin supplementation between 1-20 mg/day demonstrates a beneficial role in pediatric obesity by reducing associated mitochondrial damage, regulating glycemic homeostasis and increasing the volume/activity of brown adipose tissue. Also, no serious adverse effects were recorded (173–176). At the same time, the use of melatonin as an antioxidant has demonstrated its effectiveness in reducing muscle damage after physical activity in overweight people (177).

Although research on taurine (2-aminoethanesulfonic acid) is limited, its implications in modulating oxidative/metabolic stress, inflammation, insulin sensitivity and vascular remodeling cannot be denied. Thus, its balance can influence the appearance of the affections as well as the predisposition towards the development of associated comorbidities. For estimation purposes, the current scientific literature considers taurine excretion/24 hours inversely correlated with BMI, blood pressure profile and cholesterolemia (178–181). Also, taurine supplementation (3 g/day) doubled by sustained physical exercises, with/without nutritional counseling, demonstrated benefits in reducing inflammation, oxidative stress, preventing endothelial dysfunction induced by a high-fat diet and improving the plasticity of subcutaneous adipose tissue (182–184).

The oral and intestinal microbiota also represent a broad field of research. As I mentioned, its disturbances can be found as risk factors in various pathologies. In particular, at the local level, intestinal dysbiosis can be involved in the pathogenesis of irritable bowel, pancreatitis and celiac disease. It thus achieves a pathogenic interrelation of autoimmunities (see systemic lupus erythematosus). Therefore, the current literature states that the dietary modulation of the intestinal microbiota as well as the transplantation of fecal matter can represent prophylactic and curative strategies in childhood obesity (185–189). Its evaluation currently represents a pinnacle of research. It is known that it is in permanent change since the perinatal period, being influenced by both maternal and individual factors. The first three years are the most important for its optimal definition, the disruptive factors being cesarean birth, artificial feeding, antibiotic therapy (190, 191). Consequently, due to the multiple causal associations with obesity, it is necessary to emphasize the possible beneficial implications of prebiotics/probiotics or symbiotics in its therapeutic modulation. The ways in which they interact with the homeostasis of the internal environment playing the role of antioxidants are vast, from the clearance of reactive substances to the stimulation of signaling with the increase of the cytoprotective capacity of the host (192, 193). They have proven benefits in the co-adjuvant therapy of obesity, insulin resistance, diabetes and non-alcoholic fatty liver disease (194). The observation was demonstrated in studies on adult populations, when supplemented with *Saccharomyces boulardii* and SOD for two months. In addition, the increase in the level of vitamin D was noted (195).

Table 1 illustrates the main means of action of enzymatic and non-enzymatic antioxidants in mitigating the oxidative stress present in obesity.

4 The role of diet in obesity management

Continuing the appropriate screening of the population at risk, both the targeted therapeutic approach (pharmacological or surgical) and the adjunctive management of pediatric obesity (sleep hygiene, balanced diet, avoiding sedentary lifestyle, sustained physical exercise) is a broad topic of ongoing research. According to the current consensus, multidisciplinary management aims to prevent and counteract the underlying pathology and associated comorbidities, efforts aimed at maintaining the psychosocial integrity of the child (196–198).

In practical terms, international guidelines currently support the approach of a minimum of 60 minutes/day of moderate to vigorous physical exercises. The most effective method to eliminate excess

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TABLE 1 Means to combat oxidative stress in pediatric obesity.

The type of antioxidant	Mode of action
Enzymatic antioxidants	
Superoxide dismutase	- the first line of defense against reactive oxygen species, converting superoxide radicals into hydrogen peroxide
Catalase	- degrades hydrogen peroxide into oxygen and water
Glutathione peroxidase	- modulates the balance between necessary and harmful levels of reactive oxygen species by regulating the accumulation of hydrogen peroxide - is the main supplier of reduced glutathione, a necessary substrate for the proper development of the redox process - has a neuroprotective, anti-inflammatory effect, regulates metabolism and cardiovascular homeostasis, being also a valuable anti-oncogenic factor - its overexpression can have negative consequences
Peroxiredoxin	- catalyze the reduction of peroxides (e.g., hydrogen peroxide, organic peroxides) to water and alcohol, using thiols (e.g., thioredoxin or glutathione) as electron donors
Non-enzymatic antioxidants	
Vitamin A	- neutralizes free radicals, stabilizing them and preventing chain reactions that lead to cell damage - protects the constituent lipids of cell membranes against lipid peroxidation - modulates gene expression by binding to specific nuclear receptors, regulating the expression of genes that encode antioxidant enzymes - potentiates the effects of other antioxidants through synergy
Vitamin E	- similar to vitamin A
Vitamin C	- neutralizes free radicals through the effect of donating an electron, converting them into less reactive molecules - contributes to the regeneration of other antioxidants (e.g. vitamin E) - participates in the synthesis of collagen, an important structural and functional protein in the skin, blood vessels, bones and other connective tissues - confers protection to DNA and proteins against oxidative damage, helping to maintain the genetic and functional integrity of cells - modulates the expression of genes involved in antioxidant and anti-inflammatory responses - improves the absorption of non-heme iron from plant-based foods, helping to maintain adequate iron levels in the body
Vitamin D	- modulates through the receptor the expression of genes involved in antioxidant defense (e.g., glutathione peroxidase, superoxide dismutase, catalase) - has anti-inflammatory properties, reducing the production of pro-inflammatory cytokines (e.g., TNF-α and IL-6) - potentiates the synthesis of glutathione, one of the most important intracellular antioxidants prevents lipid peroxidation
Zinc	- contributes to maintaining the structural and functional integrity of cells, preventing lipid peroxidation - acts as a cofactor for antioxidant enzymes (e.g. superoxide dismutase with zinc and copper) inhibiting the production of free radicals - protects against DNA and protein damage modulates the inflammatory response - supports the immune system
Cooper	- plays the role of cofactor for antioxidant enzymes participates in the synthesis and functioning of cytochrome c oxidase, thus supporting mitochondrial function - supports the activity of ceruloplasmin, a transport protein with an antioxidant role (prevents the formation of free radicals through Fenton reactions) modulates the immune system - promotes the formation of collagen and elastin
Iron	- cofactor for antioxidant enzymes (e.g., catalase, peroxidase) - participate in cellular metabolism and energy production - essential for the functioning of enzymes in the electron transport chain in the mitochondrion - role in the synthesis of hemoglobin and myoglobin, proteins that transport oxygen in the blood and muscles and modulate immune responses - participates in the detoxification of free radicals
Selenium	- component of antioxidant enzymes neutralizes free radicals - participate in the regeneration of other antioxidants (e.g. vitamin C, vitamin E) - supports the immune system - *adequate levels of selenium are associated with improved immune function and increased ability to fight infections* - and reduces inflammation
Chromium	- regulates glucose and insulin levels - reduces inflammation associated with insulin resistance - supports mitochondrial function - can contribute to the activation of some antioxidant enzymes (e.g. superoxide dismutase, glutathione peroxidase)

(Continued)

TABLE 1 Continued

The type of antioxidant	Mode of action
Non-enzymatic antioxidants	
Magnesium	- neutralizes free radicals and prevents oxidative damage at the cellular level - can inhibit oxidative reactions and decrease free radical levels in cells. - is involved in the activation of essential antioxidant enzymes (e.g. superoxide dismutase and glutathione peroxidase) - supports mitochondrial function - regulates inflammation - protects DNA and proteins against oxidative damage - reduces the risk of chronic diseases
Manganese	- essential cofactor for manganese superoxide dismutase - protects cell membranes against oxidative damage through the ability to neutralize free radicals that can attack lipids in cell membranes, thus preventing lipid peroxidation - is involved in the functioning of other antioxidant enzymes (e.g., glutathione peroxidase, catalase), helping to detoxify hydrogen peroxide and other reactive oxygen species - role in glucose and lipid metabolism - supports the immune system
Flavonoids	- reduces free radical levels - it has anti-inflammatory properties and improves insulin sensitivity - they intervene in reducing fat accumulation in the body and improving lipid metabolism - intervene in the protection and regeneration of mitochondrial function
α-lipoic acid	- neutralizes a variety of free radicals and reactive oxygen species intervenes in the regeneration of other antioxidants (e.g. vitamin C, vitamin E, glutathione) - reduce inflammation - improves insulin resistance - protect mitochondria against oxidative damage and dysfunction
Coenzyme Q10	- neutralizes free radicals and reactive oxygen species - helps protect cell membranes against lipid peroxidation - is essential for the optimal functioning of mitochondria, helping to reduce the excessive production of free radicals and to prevent oxidative stress associated with mitochondrial dysfunctions - improves endothelial dysfunction - can increase the activity of other antioxidant enzymes (e.g. superoxide dismutase, glutathione peroxidase)
Melatonin	- lowers free radical levels - protects mitochondria against oxidative damage and improves their function - reduce inflammation - improves the antioxidant function of other enzymes - regulates lipid and carbohydrate metabolism, contributing to the reduction of fat accumulation and the prevention of metabolic dysfunctions associated with obesity
Taurine	- neutralizes free radicals and reduces oxidative stress - protects the liver against oxidative stress, thus reducing the risk of liver damage associated with obesity - reduce inflammation - improves insulin sensitivity - it protects the kidneys against oxidative stress and may contribute to the maintenance of renal homeostasis

weight and reduce waist circumference has proven to be the combination of aerobics and resistance training. An important factor in this approach is the school. Studies have shown that encouraging physical activity in schools has led to improvements in BMI, waist circumference in women, skinfold thickness and body fat (197, 199). Undoubtedly, in order to be effective, physical activity must be a part of changing the entire lifestyle. Thus, it was proven that the lack of breakfast, the frequent consumption of snacks, the increase in the amount of fat and carbohydrates in the diet and a narrow variability of healthy food options (e.g., fruits, vegetables and dairy) were precipitating factors of childhood obesity. Added to these are the consumption of sugar-sweetened beverages or fast food (199–201).

The pharmacological intervention possibilities are more restrictive compared to those in the adult population. The main consideration resides in the lack of substance certification studies. Therefore, the pharmacological substances currently approved for obesity are Orlestin, glucagon-like peptide-1 analogues, Liraglutide, Setmelanotide and Metreleptin. The means of action, dosage and indications are varied, thereby ensuring the possibility of an individualized therapy. We also note Semaglutide and Exenatide as therapeutic options in research regarding the utility in obesity (199).

Bariatric surgery represents a therapeutic alternative dedicated to severe cases of obesity, refractory to the previously described hygienic-dietary and pharmacological measures and which associate comorbidities. Its indications are defined depending on the value of the body mass index. We thus recognize the usefulness of bariatric surgery when BMI >40 kg/m2 or BMI >35 kg/m2 with significant comorbidities. The possible surgical techniques are quite varied, and BMI reduction seems to be effective in a follow-up interval of 1-5 years post-procedural. Additionally, this has proven

TABLE 2 Food sources rich in antioxidants (adapted from Webster-Gandy J. et al., Kozłowska A. et al., Alam MA. et al., Meng X. et al., Wu G. et al. and Shay KP. et al.) (165, 204–208).

Antioxidants	Food
Vitamins A	• Liver and liver products; • Kidney and offal; • Oily fish and fish liver oils; • Eggs; • Carrots; • Red peppers; • Spinach; • Broccoli; • Tomatoes.
E	• Wheat germ oil; • Almonds; • Sunflower seeds & oil; • Safflower oil; • Hazelnuts; • Peanuts & peanut butter; • Corn oil.
C	• Kiwi fruit; • Citrus fruit (oranges, lemons, satsumas, clementines, etc.); • Black currants; • Guava; • Mango; • Papaya; • Pepper; • Brussels sprouts; • Broccoli; • Sweet potato.
D	• Cod liver oil; • Oily fish (salmon, mackerel, etc.); • Milk; • Margarine; • Breakfast cereals; • Eggs; • Liver.
Microelements Zinc	• Lamb; • Leafy & root vegetables; • Crabs & shellfish; • Beef; • Offal; • Whole grains; • Pork; • Poultry; • Milk and milk products; • Eggs; • Nuts.
Copper	• Offal; • Nuts; • Cereals & cereal products; • Meat & meat products.
Iron	• Meat especially offal; • Fish; • Eggs; • Meat extracts; • Bread & flour; • Breakfast cereals; • Vegetables (dark green) & pulses; • Nuts & dried fruit—prunes, figs, apricots; • Yeast extract.
Magnesium	• Green vegetables; • Pulses & whole grain cereals; • Meats.
Selenium	• Offal; • Fish; • Brazil nuts; • Eggs; • Poultry; • Meat and meat products.
Chromium	• Meat; • Whole grains; • Legumes; • Nuts.
Manganese	• Cereals & cereal products; • Tea; • Vegetables.
Other antioxidants Flavonoids	• Fruits; • Vegetables; • Nuts; • Seeds; • Spices.
α-lipoic acid	• Muscle meats; • Heart; • Kidney; • Liver.
Coenzyme Q10	• Meat; • Poultry; • Fish.
Melatonin	• Eggs; • Over; • Cereals (corn, rice); • Fruits (grapes, strawberries); • Vegetables (tomatoes, peppers, mushrooms); • Seeds (white and black mustard, soy, flax, beans); • Nuts (pistachio); • Coffee; • Balsamic vinegar; • Extra virgin olive oil.
Taurine	• Beef.

to be beneficial in improving associated comorbidities (e.g., diabetes, hypertension, dyslipidemia, and proteinuria). The studies that will unequivocally certify the role of bariatric surgery and the superiority of each technique are currently underway (199, 201). One such example is a multicenter study undertaken by Järvholm K. et al. (202).

Finally, in accordance with what was stated previously, Stabouli S. et al. support the need for future research on the early detection of risk factors and the elucidation of the mechanisms underlying pediatric obesity (203). Of these, we detailed throughout the manuscript the pathophysiology implications of antioxidant substances in the evolutionary pattern of obesity. Therefore, in order to add practical utility to the work, Table 2 refers to the main foods with a high content of antioxidants, beneficial in the diet of children and obese adolescents.

5 Conclusions

Obesity is found both in childhood/adolescence and in adulthood. Many pathological processes compete with its induction and maintenance. Therefore, the unbalanced lifestyle is only the tip of the iceberg. The current work achieved its goal of bringing the concept of pediatric obesity and accompanying comorbidities up to date. Most of the obese children, without therapeutic interventions, will evolve further into obese adults that can also associate metabolic syndrome. In order to improve/stop the systemic decline, measures aimed at lifestyle modification, pharmacotherapy or surgical therapy are considered. Knowing the impact played by released free radicals as a consequence of oxidative stress, we discussed the main endogenous or exogenous antioxidant substances that interfere with the pathological process, briefly detailing the roles of each. Although the implications of antioxidants in childhood obesity are certified by specialized medical literature, we identified in our search a reporting bias of the main food sources rich in substances with an antioxidant role. Consequently, we chose to do a brief review of them. In conclusion, the current study places the obesity-oxidative stress-antioxidants relationship in a different light. We consider it opportune to widen the horizons in this direction by designing and implementing screening programs and individualized supplementation of nutritional deficiencies in antioxidant substances with the aim of reducing the incidence and burden of pediatric obesity.

Author contributions

AL: Conceptualization, Investigation, Writing – original draft. SF: Investigation, Methodology, Writing – original draft. EJ: Investigation, Methodology, Writing – original draft. IS: Investigation, Software, Writing – original draft. II: Investigation, Software, Writing – original draft. AK: Validation, Visualization, Writing – review & editing. DS: Software, Validation, Writing – review & editing. MS: Validation, Visualization, Writing – review & editing. OC: Investigation, Software, Writing – original draft. NR: Supervision, Validation, Writing – review & editing. CMM: Writing – review & editing, Visualization. VL: Methodology, Supervision, Writing – review & editing. AN: Project administration, Validation, Writing – review & editing.

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