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Taste and healthy eating in the context of well-being, sustainability and 21st century food science

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Taste and healthy eating in the context of well-being, sustainability and 21st century food science

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Global trends and research hotspots of EAT-Lancet diet: a bibliometric analysis

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The EAT-Lancet diet is a groundbreaking and comprehensive dietary framework that has garnered significant attention in the fields of nutrition, sustainability, and public health. We aimed to conduct a bibliometric study to investigate current status and hotspots in the field of EAT-Lancet diet based on the Web of Science Core Collection (WOSCC) database, and the documents of EAT-Lancet diet published from Jan 1, 2019 to Sep 1.2023 were extracted. The bibliometric and visualized analysis were performed by VOSviewer 1.6.16 and WOSCC Online Analysis Platform. In total, 155 documents from 62 journals were included, and 735 authors from 389 institutions and 53 countries/regions contributed to the field of EAT-Lancet diet. The most productive countries/regions, institutions, authors, and journals were the USA, Wageningen University & Research, Johan Rockström, and Nutrients, respectively. The first high-cited document was published in Lancet and authored by Willett et al. in 2019. This is also the first study about EAT-Lancet diet. The article firstly proposed the "EAT-Lancet Diet" emphasizing balanced, plant-based eating to improve human health while addressing environmental concerns. In conclusion, in the field of EAT-Lancet diet, the main research hotspots and frontiers are the adaptation of EAT-Lancet diet, the composition of EAT-Lancet diet, and the benefits of EAT-Lancet diet for human health. The number of research on the EAT-Lancet diet is currently limited. There is a pressing need for further studies to broaden our understanding of the EAT-Lancet diet and its potential to enhance human health.

KEYWORDS

EAT-lancet diet, bibliometric analysis, research hotspots, human health, adaptation

Introduction

The EAT-Lancet diet, also known as the "planetary health diet," is a groundbreaking and comprehensive dietary framework that has garnered significant attention in the fields of nutrition, sustainability, and public health. Proposed by the EAT-Lancet Commission, a collaboration of experts from around the world, this diet is designed to address two interconnected global challenges: optimizing human health and promoting environmental sustainability (1–5). The EAT-Lancet diet emphasizes a profound shift in our eating habits to achieve a more sustainable and health-conscious way of nourishing ourselves. It goes beyond the traditional understanding of dietary guidelines by considering not only the nutritional needs of individuals but also the environmental impact of our food choices (6–8). This revolutionary dietary approach calls for a significant increase in the consumption of plant-based foods, such as whole grains, fruits, vegetables, legumes, and nuts. It encourages a reduction in the intake of animal-based products, particularly red meat, while allowing moderate amounts of fish and poultry. Added sugars, refined grains, and unhealthy fats are minimized in favor of healthier fats from sources like avocados and nuts. What truly sets the EAT-Lancet diet apart is its dual focus on human well-being and the planet's health. It recognizes the urgent need to transform our global food systems to align with the United Nations' Sustainable Development Goals (SDGs) and the Paris Agreement. By adopting this dietary approach, it is believed that we can not only improve individual health but also mitigate the environmental challenges posed by agriculture, including climate change, biodiversity loss, and resource depletion. Moreover, the EAT-Lancet diet is designed to be flexible, adaptable to diverse cultural, economic, and environmental contexts worldwide. It offers a blueprint for healthier eating patterns that can be tailored to local traditions and preferences. The EAT-Lancet diet represents a visionary response to the complex and interconnected issues of nutrition and sustainability. It offers a path toward healthier lives for individuals and a more sustainable future for our planet, making it a topic of great importance and interest in the realms of nutrition, public health, and environmental science (4, 9–13).

The EAT-Lancet diet has garnered significant interest. However, despite the importance of this topic, a comprehensive bibliometric analysis is lacking, one that encapsulates existing publication patterns and forecasts emerging research focal points in this domain. Bibliometric analysis is a systematic and quantitative method used to assess and analyze the scholarly literature related to a specific research topic (14–18). It involves the collection and evaluation of bibliographic data, such as publications, citations, authors, journals, and institutions, to gain insights into the research trends, knowledge networks, and impact of this dietary framework. By employing bibliometric techniques, researchers can uncover key patterns and dynamics in the academic discourse surrounding the EAT-Lancet diet, identify influential authors and institutions, track the evolution of research themes over time, and discern emerging areas of interest and research gaps. This approach may be helpful to both academics and policymakers seeking a comprehensive understanding of the scientific landscape and the dissemination of knowledge in the realm of sustainable and healthy diets. Consequently, our study embarked on a bibliometric evaluation with the objective of identifying the leading edges and hotspots within the realm of the EAT-Lancet diet.

Methods

Data sources and search strategies

The primary literature source for this bibliometric analysis was the Web of Science Core Collection (WoSCC), renowned for its comprehensive and authoritative collection of research publications. The specific search terms utilized were (EAT-Lancet OR "planetary health diet" OR "planetary diet"). As the first document about the EAT-Lancet diet was published in 2019 (2), the search results were confined by the publication date from Jan 1, 2019 to Sep 1.2023; with all types of publications related to EAT-Lancet diet, and with no language restriction.

Bibliometric analysis and visualization

Once the relevant literature was identified and selected, several metrics were analyzed using the WoSCC Online Analysis Platform. This included the annual number of publications, a ranking of the top 10 most productive countries/regions, institutions, authors, and journals, and an identification of the top 20 highly-cited articles related to the "EAT-Lancet" topic. For further in-depth analysis, the data, encompassing details about publication years, authors, countries, regions, institutions, journals, keywords, and references, was downloaded in TXT format with the "Full Record and Cited References" option. Subsequently, VOSviewer software (version 1.6.16) was utilized to visualize various data sets, including co-authorship networks (spanning institutions, countries/regions, and individual authors), citation patterns of journals and references, co-citation networks, and keyword co-occurrence networks. Upon completion, pertinent visualizations were exported to support the findings of the analysis. In the keyword co-occurrence analysis, we merged the synonyms of "sustainable diet" to "sustainable diets" and "impact" to "impacts."

Results

Trends in global publications

Based on the provided visual data, a total of 155 documents concerning the topic "EAT-Lancet" were identified in the bibliometric study after screening, which is shown in Figure 1. The breakdown of the types of documents as visualized in Figure 2A, suggests that there were 104 articles (67.1%), 31 reviews (20%), and 20 other types of publications (12.9%). For the subject areas where the documents were primarily published, Figure 2B provides a detailed breakdown. The leading subject category was "Nutrition Dietetics" with 61 documents, accounting for 39.35% of the total publications. This was followed by "Food Science Technology" with 27 documents, representing 17.42%, and "Environmental Sciences" with 26 documents, or 16.67%. Regarding the temporal distribution of publications, Figure 2C depicts the trend from 2019 to 2023. The growth in the number of publications on the "EAT-Lancet" topic is evident, starting with 10 in 2019, more than doubling to 31 in 2020, followed by a consistent rise to 34 in 2021, 44 in 2022, and reaching 36 in 2023. The dotted line in the figure indicates an overall positive trend in the number of publications over the years, emphasizing the increasing importance and recognition of the "EAT-Lancet" topic in the academic and scientific community.

Analysis of countries or regions, institutions, and authors, journals

In total, 735 authors from 389 institutions and 53 countries/regions contributed to the field of EAT-Lancet diet in 62 journals. On the country front, the USA leads the contribution with 45 publications,

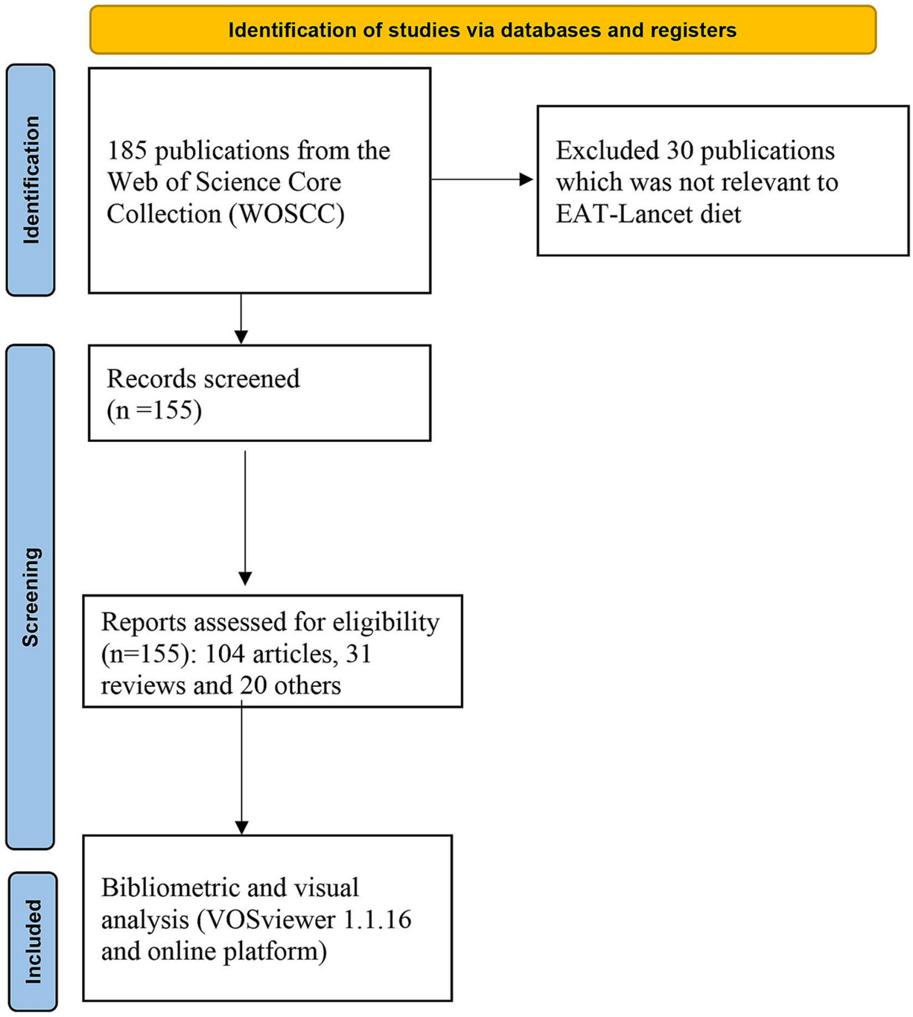

FIGURE 1
Flow search chart.

gathering a considerable 5,146 citations and an H-index of 17, reflecting both volume and the impact of the research. Following the USA, England with 28 publications and the Netherlands with 23 have been active contributors. While the USA and England are expected leaders given their robust research ecosystems, the significant contribution from the Netherlands suggests a keen interest in the topic regionally. Wageningen University & Research stands out as the top institution in this field, with 11 publications, followed closely by globally renowned institutions like Harvard University with 10 publications and the University of Oxford with 9. When it comes to individual contributors, Johan Rockström tops the list with 6 publications and an impressive 4,046 citations. Ulrika Ericson and Leandro Teixeira Cacau also appear prominent with each having 6 publications. Notably, while Johan Rockström has the same number of publications as Ulrika and Leandro, his citation count is considerably higher. The top three journals that published the most on the EAT-Lancet topic are "Nutrients" from Switzerland with 17 publications, "Lancet" from England boasting a significant 4,130 publications, and "Nature Food" from England with 10 publications. In summary, the EAT-Lancet topic has garnered significant attention from prestigious journals, leading countries in research, globally renowned institutions, and acclaimed researchers. The data underscores the topic's relevance and the collaborative nature of the research spanning across multiple countries and institutions. The most productive authors, institutions, and countries/regions in the field of EAT-Lancet diet are summarized in Table 1. The network visualization maps of citations of journals, countries/regions, institutions, and authors, are shown in Figures 3, 4 (see Table 2).

Analysis of highly-cited and co-cited publications

The network visualization maps of citations and co-cited documents are displayed in Figure 5. The attributes of the 20 most-cited publications are encapsulated in Table 3 (1–3, 6, 8, 19–33). The first high-cited document was published in Lancet and authored by Willett et al. (2). This is also the first study about EAT-Lancet diet. The article firstly proposed the "EAT-Lancet Diet" emphasizing balanced, plant-based eating to improve human health while addressing environmental concerns. It stresses the need for transforming food systems, considering global and regional perspectives, reducing

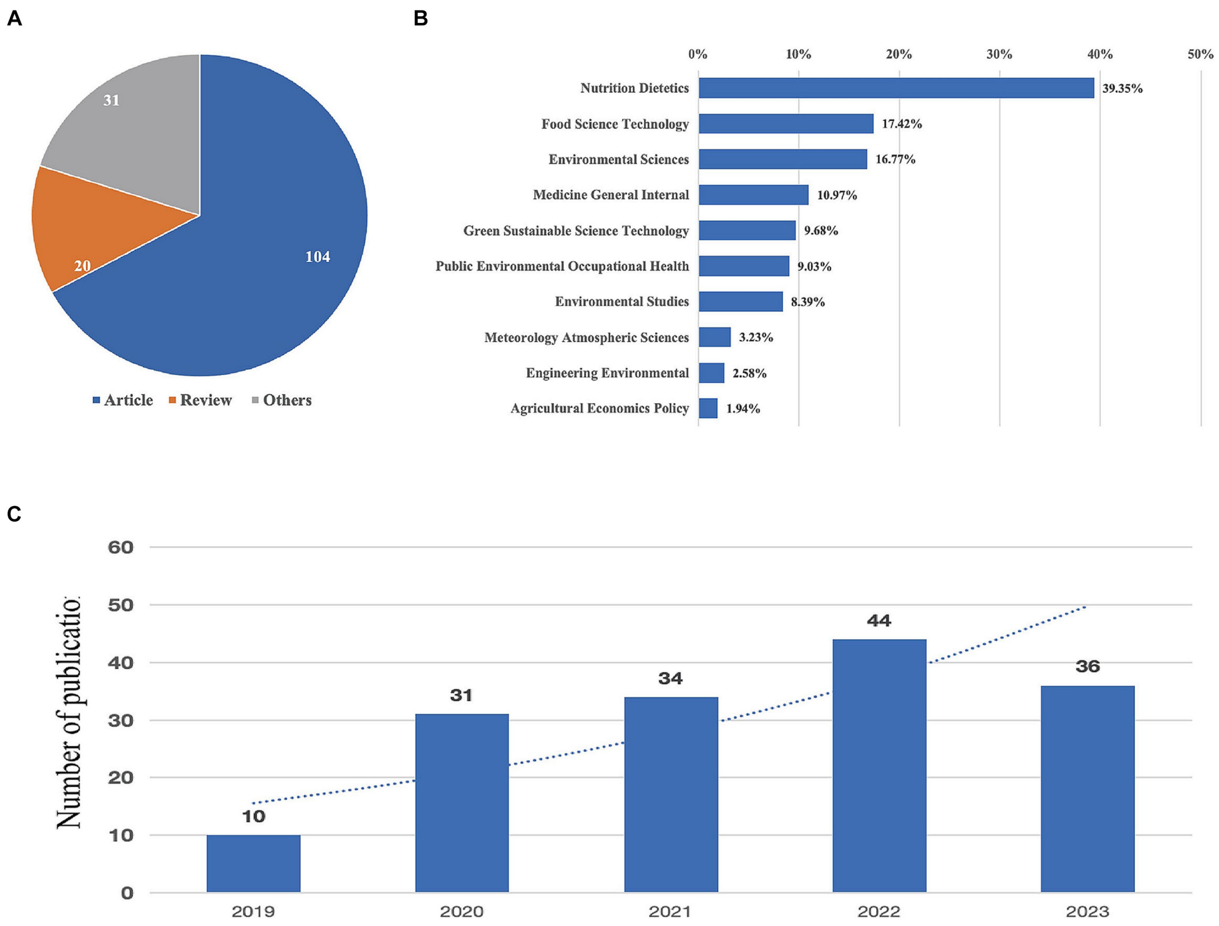

FIGURE 2
Yearly quantity and literature type of publications.

environmental impacts, promoting food equity, and implementing policy changes for a more sustainable and nutritious future. The second high-cited publication was published in Lancet Global Health by Hirvonen et al. (21). They conducted a global analysis of the affordability of the EAT-Lancet reference diet. The EAT-Lancet reference diet is a dietary framework designed to promote both human health and environmental sustainability. It emphasizes a balanced diet with a focus on plant-based foods while limiting the consumption of red meat and sugar. The research aimed to assess whether the EAT-Lancet reference diet is affordable for people in different regions around the world. Affordability is a crucial factor in determining whether individuals and households can access and adopt a particular dietary pattern. Key findings and implications from the study may include insights into the affordability of the EAT-Lancet reference diet across various countries and income levels. It can inform discussions and policy considerations related to promoting sustainable and healthy diets globally. The third high-cited publication was published in BMJ and authored by Springmann et al. (28). They performed a modeling analysis that focuses on evaluating the healthiness and sustainability of national and global food-based dietary guidelines (FBDGs). FBDGs are recommendations provided by governments and health organizations regarding what individuals and populations should eat for optimal health. The research uses mathematical models to assess the nutritional quality of dietary guidelines from various countries and regions, including the EAT-Lancet diet, considering factors such as their impact on human health and their environmental sustainability. The study aims to provide insights into whether these guidelines are aligned with both health and sustainability goals. Key findings and implications from the study may include assessments of the strengths and weaknesses of different dietary guidelines and recommendations for improving the alignment of FBDGs with health and sustainability objectives. The fourth high-cited publication was published in Global Food Security and authored by Adesogan et al. (19). They explore the role of animal source foods in the context of sustainability and nutrition. It emphasizes that perspectives on this issue vary and depend on one's viewpoint. In the context of the EAT-Lancet diet, which encourages a shift towards plant-based eating for sustainability reasons, this article may provide an alternative perspective. It suggests that animal source foods can play a role in addressing malnutrition and may be part of a sustainable food system when managed responsibly. However, the article acknowledges that different viewpoints exist and highlights the importance of considering various perspectives when discussing the role of animal source foods in diets and sustainability. The fifth high-cited publication was published in Frontiers in Nutrition and authored by Obeid et al. (25). They explore the important role of vitamin B12 intake primarily from animal-based foods. In the context of the EAT-Lancet diet, which emphasizes a reduction in animal product

TABLE 1 The top productive authors, institutions and countries based on publications.

Items	Publications			
	Ranking	Country	Number	Citations
Country	1	USA	45	5,146
	2	England	28	4,552
	3	Netherlands	23	4,369
	4	Italy	20	4,281
	5	Germany	15	4,167
	6	Sweden	14	4,178
	7	Australia	12	4,300
	8	France	12	4,136
	9	Peoples R China	12	84
	10	Denmark	10	122
Institution	1	Wageningen University & Research	11	4,213
	2	Harvard university	10	4,273
	3	University of Oxford	9	4,386
	4	CGIAR	8	4,381
	5	Instituto Nacional De Salud Publica	8	4,082
	6	Johns Hopkins University	8	4,126
	7	Universidade De São Paulo	7	57
Author	1	Johan Rockström	6	4,046
	2	Ulrika Ericson	6	57
	3	Leandro Teixeira Cacau	6	56
	4	Brent Loken	6	4,046
	5	Dirce Maria Marchioni	6	56
	6	Anna Stubbendorff	6	57
	7	Walter Willett	5	4,072
	8	Juan A Rivera	5	4,079

consumption for sustainability reasons, this article provides insights into the potential health implications of lower vitamin B12 intake. Vitamin B12 is primarily found in animal products, and reduced consumption of these foods can lead to vitamin B12 deficiency, which has health implications. The article likely discusses the importance of monitoring vitamin B12 levels and the potential need for supplementation or alternative dietary sources in plant-based diets to ensure adequate intake and avoid health issues associated with deficiency. It highlights the complexity of dietary choices and their impact on nutrient intake and health, especially when considering diets that limit animal-based foods like those promoted by the EAT-Lancet diet. For the co-cited documents, the top 2 highest co-cited studies were consistent with the highest cited publications. The third highest co-cited document was published in Lancet by Afthin et al. in 2019 (34). In this systematic analysis, they investigated the influence of poor diets on non-communicable diseases (NCDs) across 195 countries. Utilizing a comparative risk assessment method, it was found that in 2017, dietary risk factors contributed to 11 million deaths and 255 million disability-adjusted life-years (DALYs). The leading dietary culprits included high sodium intake, insufficient whole grains, and low fruit consumption. Despite some data uncertainties due to varied sources, the study emphasizes the critical role of diet in global health and underscores the need for evidence-based dietary interventions. The fourth highest co-cited document was published in Lancet by knuppel et al. (1). It is the first study about EAT-Lancet diet and main health outcomes. In this study conducted on 46,069 participants from the UK involved in the EPIC-Oxford research, the EAT-Lancet Commission's 2019 universal reference diet, aimed at promoting human and environmental health, was evaluated. Using food frequency questionnaires from 1993 to 2001, participants were scored between 0–14 based on their adherence to 14 key dietary recommendations. Results indicated that high adherence to the EAT-Lancet score corresponded to a 28% reduced risk of ischemic heart disease and a 59% reduced risk of diabetes. However, there was no clear link between the score and the risk of stroke or overall mortality. The study suggests that the diet's benefits are cumulative from multiple recommendations, with the majority of participants adhering to recommendations for poultry, eggs, fish, legumes, and fats. The high adherence might also reflect the cohort's large vegetarian population and could be a marker for an overall healthy lifestyle. The fifth highest co-cited document was published in science by Springmann in 2020 (35). Their research reveals significant variation, up to 50-fold, in environmental impact among producers of the same product, suggesting opportunities for environmental mitigation. However, achieving these reductions is complex due to trade-offs, diverse methods available to producers, and supply chain interactions. Interestingly, even the lowest-impact animal products tend to have greater environmental impacts than vegetable substitutes, emphasizing the potential environmental benefits of dietary shifts. The study advocates for producers to actively monitor and communicate their environmental impacts, and to flexibly choose from various practices to meet environmental goals.

Analysis of keywords

Figure 6 displayed the network visualization maps of co-occurrence of keywords, which could be classified in four clusters, including: Health impacts and ASPECTS that diets exert on human well-being. This domain covers the gamut from generic health and diet quality to specific ailments like coronary heart diseases and malnutrition. Dietary practices and behaviors, which delve into consumption patterns, food frequency, and tools to measure these behaviors such as the food frequency questionnaire. Dietary patterns and types that include prominent diets like the Mediterranean and Eat-Lancet diets, along with broader themes like dietary guidelines, patterns, and the evolving concept of sustainable diets. Intertwined with these dietary considerations are pressing

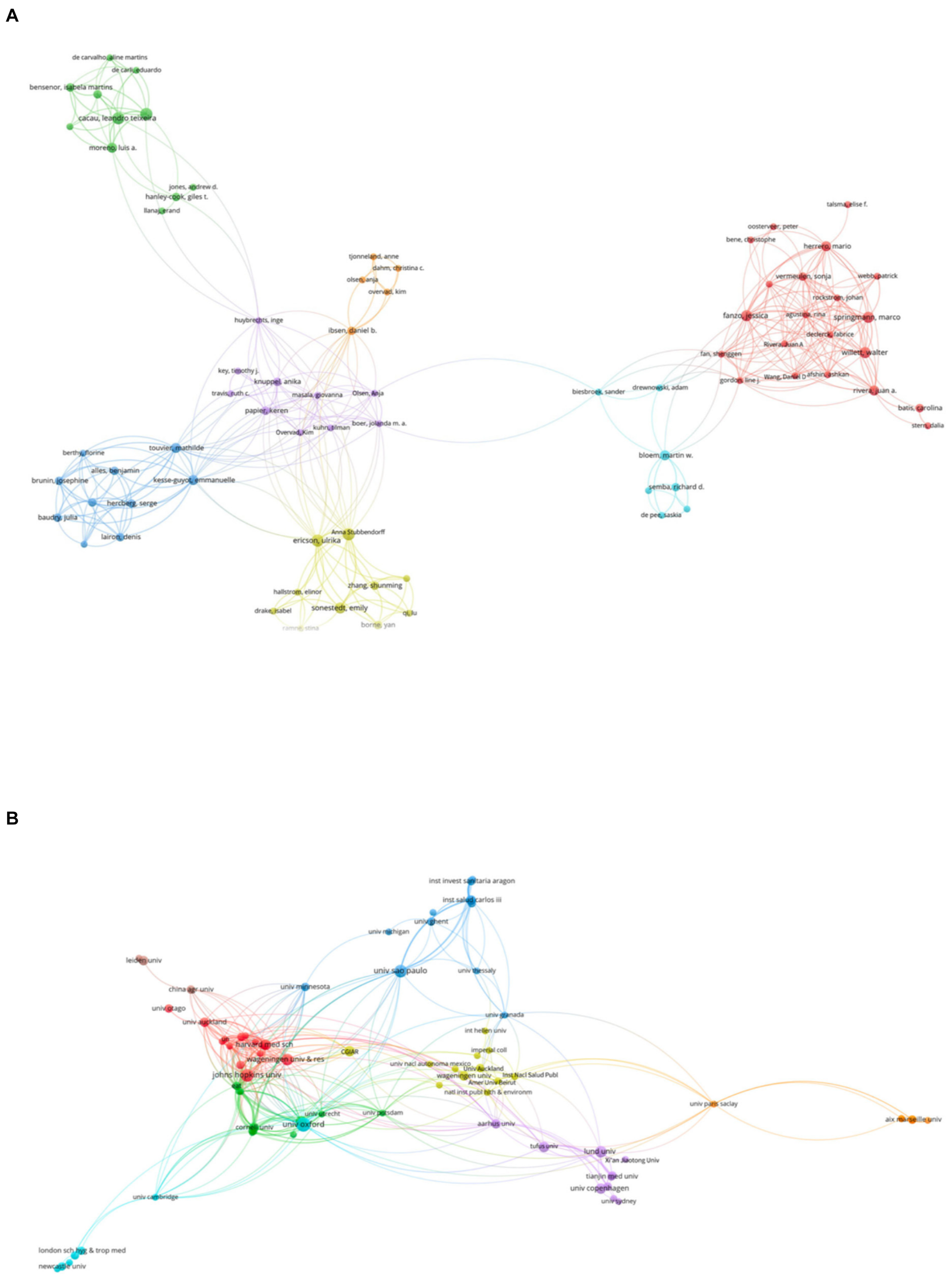

FIGURE 3
Visualization knowledge maps of authors and institutions.

Environmental Aspects. Key points here encapsulate the environmental impact of dietary choices, highlighting metrics like the carbon and water footprints and the overarching shadow of climate change. Sustainability and global issues emerge as critical, shedding light on the symbiosis between planetary health, food security, and nutritional adequacy.

Discussion

General information

To the best of our knowledge, this is the first comprehensive bibliometric analysis of the EAT-Lancet diet. The EAT-Lancet diet

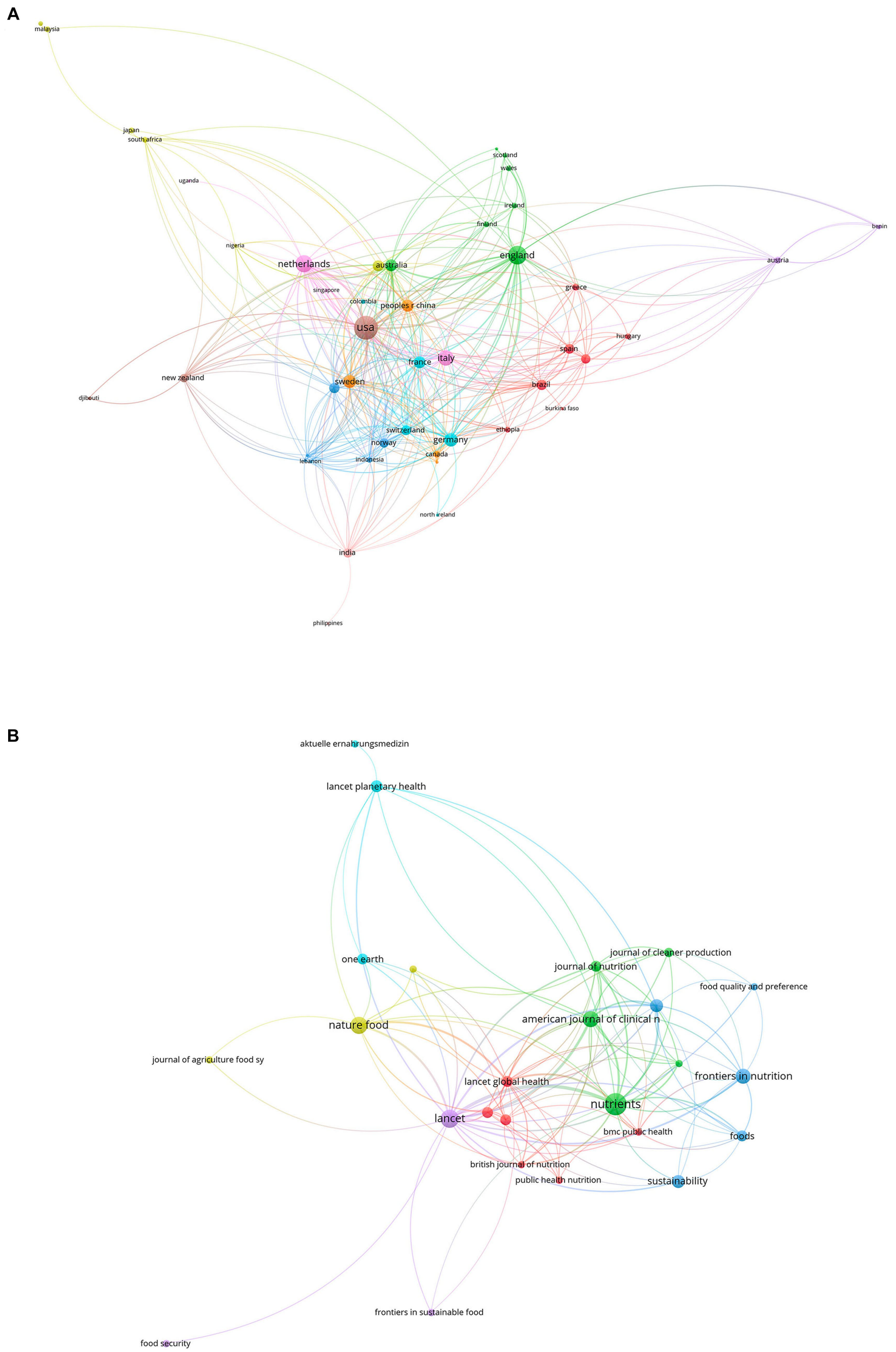

FIGURE 4
The visualization knowledge map of countries/regions and journals.

TABLE 2 The top productive journals.

Ranking	Journal name	Country	Counts	Citation
1	Nutrients	Switzerland	17	198
2	Lancet	England	11	4,130
3	Nature Food	England	10	173
4	American Journal of Clinical Nutrition	USA	9	78
5	Frontiers in Nutrition	Switzerland	8	183
6	European Journal of Nutrition	Germany	6	20
7	Sustainability	Switzerland	6	71
8	Lancet Planetary Health	England	5	54

represents a transformative step in the field of nutrition, sustainability, and global food systems. It emerged as a critical topic of research and discourse, seeking to address both human health and planetary health, aiming to devise a dietary pattern that would not only optimize health outcomes but also reduce the environmental footprint of our food choices. The data analysis sheds light on the considerable attention the EAT-Lancet topic has garnered from the research community. In our bibliometric analysis, a total of 155 publications addressing the EAT-Lancet diet span across prestigious journals such as "Nutrients," "Lancet," and "Nature Food." The USA leads the global contributions with 45 publications, reflecting the nation's pivotal role in advancing research on this topic. Notably, 53 countries have made significant contributions, indicating a broad international interest. From an institutional perspective, Wageningen University & Research has been at the forefront, closely followed by globally acclaimed institutions like Harvard University and the University of Oxford. These institutions have not just contributed in volume but have also made impactful contributions as denoted by their high citation counts. On the individual front, Johan Rockström stands out with 6 publications, amassing an impressive 4,046 citations, indicating the broad reach and influence of his work. A pivotal publication from Lancet, authored by Willett et al. deserves special mention. This work was the first to propose the concept of the "EAT-Lancet Diet." It brought forth the idea of a balanced, primarily plant-based diet aimed at improving human health while simultaneously addressing environmental sustainability. The article emphasized the urgent need for a shift in global food systems, considering both global and regional perspectives. It called for reduced environmental impacts, the promotion of food equity, and the importance of implementing policy changes to drive a sustainable and nutritious future. In essence, the EAT-Lancet diet topic encapsulates a holistic vision of the future of food - one that intertwines human health, environmental sustainability, and global collaboration. The significant contributions from leading countries, institutions, and researchers underscore the topic's global relevance and the collaborative nature of the research.

Hotspots and frontiers

On the basis of publications of EAT-Lancet diet, highly-cited publications, and important keywords with high frequency, the research hotspots in the field of EAT-Lancet diet were summarized as follows:

The adaptation of EAT-Lancet diet

Adapting the EAT-Lancet diet to diverse cultural and regional contexts is a hotspot (22, 36–41). Traditional diets are deeply rooted in cultural practices, and a one-size-fits-all approach may not be feasible. Customizing the diet to align with cultural preferences and food availability while maintaining sustainability goals is a challenge. Many studies aimed to develop different types of diet based on EAT-Lancet diet to adapt to the needs of different cultures and regions. For example, in a study comparing the environmental impacts of two dietary patterns (42), the Italian-Mediterranean (EAT-IT) based on the "Planetary diet" and the Italian Dietary Guidelines (IDG), it was found that the EAT-IT diet had a significantly lower carbon footprint (*CF*) than the IDG. However, there was no notable difference in their water footprints (WF). Protein-rich foods were major contributors to both *CF* and WF in both diets. Environmental outcomes were further impacted by choices like opting for frozen over fresh foods, imported fruits over local ones, greenhouse vegetables over seasonal varieties, and processed legume foods over unprocessed legumes. The EAT-IT diet is more carbon sustainable, but individual food choices significantly affect its environmental footprint. Lassen et al. (22) aimed to design a healthy plant-based diet tailored for Denmark using the global EAT-Lancet reference diet. Initially, the EAT-Lancet diet was adjusted based on available Danish foods. It was then further refined to match national dietary guidelines and current consumption trends, incorporating processed foods, discretionary items, and beverages. This adapted diet met most nutrient recommendations for individuals aged 6–65 years, excluding vitamin D and iodine. The research highlighted the importance of emphasizing legumes, nuts, seeds, fruits, dark green vegetables, whole grains, and vegetable oils, while reducing meat consumption, offering guidance for future sustainable dietary guidelines. Nomura et al. assessed the Japanese diet in relation to the global EAT-Lancet Commission's Planetary Health Diet (PHD), particularly focusing on protein intake across various age groups. Using data from the Japan National Health and Nutrition Survey 2019 (43), it was found that while most food group intakes exceeded the PHD's global reference across all age groups, only red meat intake significantly surpassed its upper limit, particularly in those in their 40s. Despite this, protein consumption in Japan remains within recommended limits. As seen in various Western regions, the Japanese diet has an excessive red meat intake compared to the PHD's reference. However, the overall protein intake is within the national guidelines. The study underscores the potential of the PHD as an eco-friendly and healthy option for Japan's aging population. It highlights the need for sustainable dietary guidelines, better nutrition education, and an environment that promotes healthier food choices.

The composition of EAT-Lancet diet

A key advantage of the EAT-Lancet diet is its emphasis on sustainability (44–50). By promoting reduced meat consumption and a shift towards plant-based foods, it aims to reduce greenhouse gas

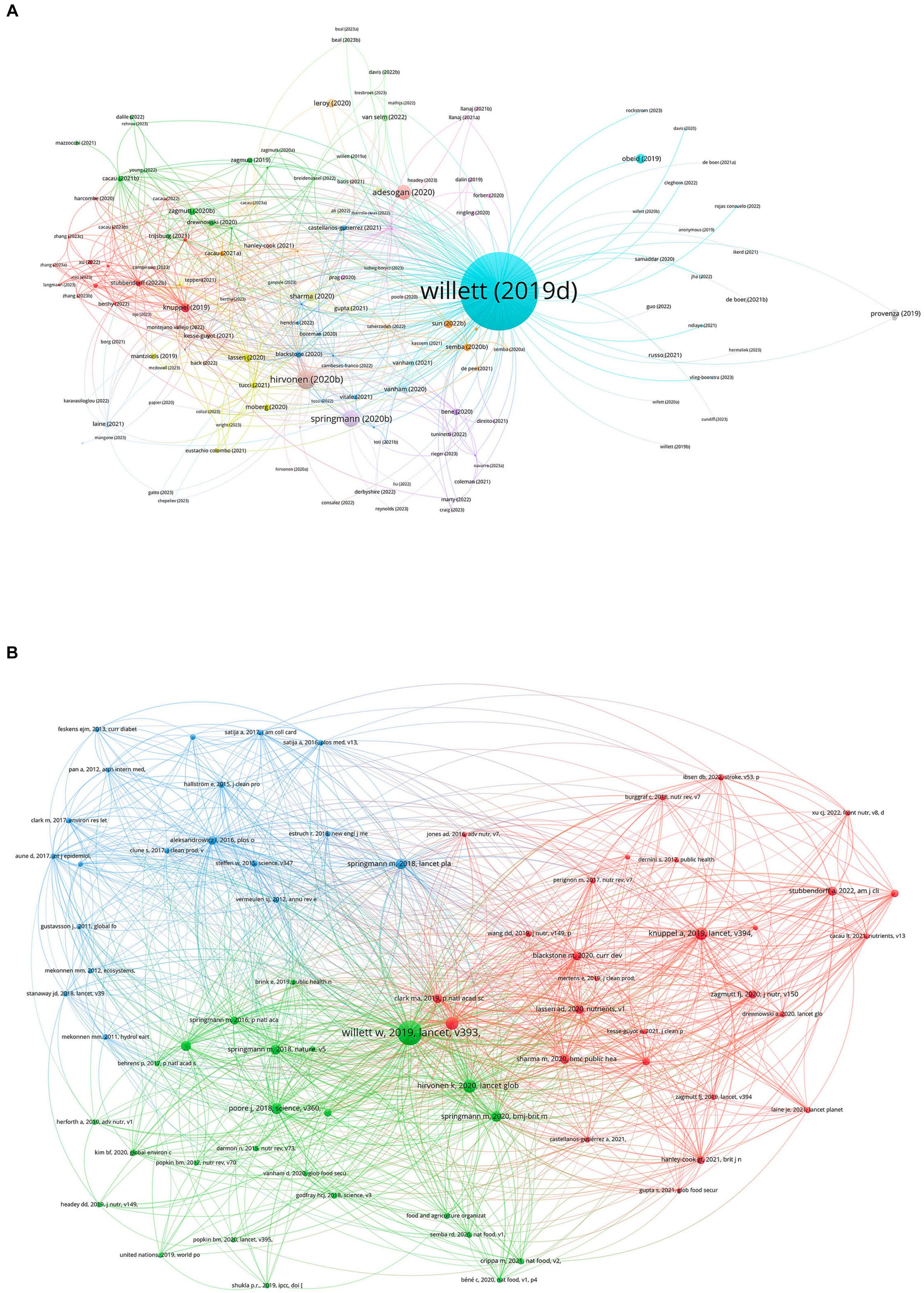

FIGURE 5
The visualized citations of highly-cited and co-cited publications.

emissions, land use, and water use associated with food production. The diet is rich in vegetables, fruits, whole grains, legumes, and nuts. Such a nutrient-rich diet can help reduce the risk of chronic diseases like heart disease, diabetes, and certain cancers. It recommends diverse food sources which can help address global issues of not just overnutrition (like obesity) but also undernutrition. However, some

TABLE 3 The top 20 most highly cited references.

Rank	Title	Journal	Citations	Year	First author
1	Food in the Anthropocene: the EAT-Lancet Commission on healthy diets from sustainable food systems	Lancet	4,040	2019	Walter Willett
2	Affordability of the EAT-Lancet reference diet: a global analysis	Lancet Global Health	225	2020	Kalle Hirvonen
3	The healthiness and sustainability of national and global food based dietary guidelines: modelling study	BMJ	195	2020	Marco Springmann
4	Animal source foods: Sustainability problem or malnutrition and sustainability solution? Perspective matters	Global Food Security	146	2020	Adegbola Tolulope Adesogan
5	Vitamin B12 Intake from Animal Foods, Biomarkers, and Health Aspects	Frontiers In Nutrition	68	2019	Rima Obeid
6	A comparison of the Indian diet with the EAT-Lancet reference diet	Bmc Public Health	67	2020	Manika Sharma
7	EAT-Lancet score and major health outcomes: the EPIC-Oxford study	Lancet	56	2019	Anika Knuppel
8	Should dietary guidelines recommend low red meat intake?	Critical Reviews in Food Science And Nutrition	54	2020	Frédéric Leroy
9	Dietary change in high-income nations alone can lead to substantial double climate dividend	Nature Food	48	2022	Zhongxiao Sun
10	Development of a Danish Adapted Healthy Plant-Based Diet Based on the EAT-Lancet Reference Diet	Nutrients	47	2020	Anne D Lassen
11	Bambara Groundnut: An Underutilized Leguminous Crop for Global Food Security and Nutrition	Frontiers In Nutrition	41	2020	Xin Lin Tan
12	Benchmarking the Swedish Diet Relative to Global and National Environmental Targets-Identification of Indicator Limitations and Data Gaps	Sustainability	35	2020	Emma Moberg
13	Circularity in animal production requires a change in the EAT-Lancet diet in Europe	Nature Food	34	2022	Benjamin van Selm
14	Adoption of the 'planetary health diet' has different impacts on countries' greenhouse gas emissions	Nature Food	34	2020	Richard D Semba
15	The EAT-Lancet Commission's Dietary Composition May Not Prevent Noncommunicable Disease Mortality	Journal of Nutrition	34	2020	Francisco J Zagmutt
16	Treenuts and groundnuts in the EAT -Lancet reference diet: Concerns regarding sustainable water use	Global Food Security	34	2020	Davy Vanham
17	Five priorities to operationalize the EAT-Lancet Commission report	Nature Food	31	2020	Christophe Béné
18	Co-benefits from sustainable dietary shifts for population and environmental health: an assessment from a large European cohort study	Lancet Planetary Health	30	2021	Laine JE
19	Comparing the Recommended Eating Patterns of the EAT-Lancet Commission and Dietary Guidelines for Americans: Implications for Sustainable Nutrition	Current Developments In Nutrition	28	2020	Nicole Tichenor Blackstone
20	Analyzing the affordability of the EAT-Lancet diet	Lancet Global Health	28	2020	Adam Drewnowski

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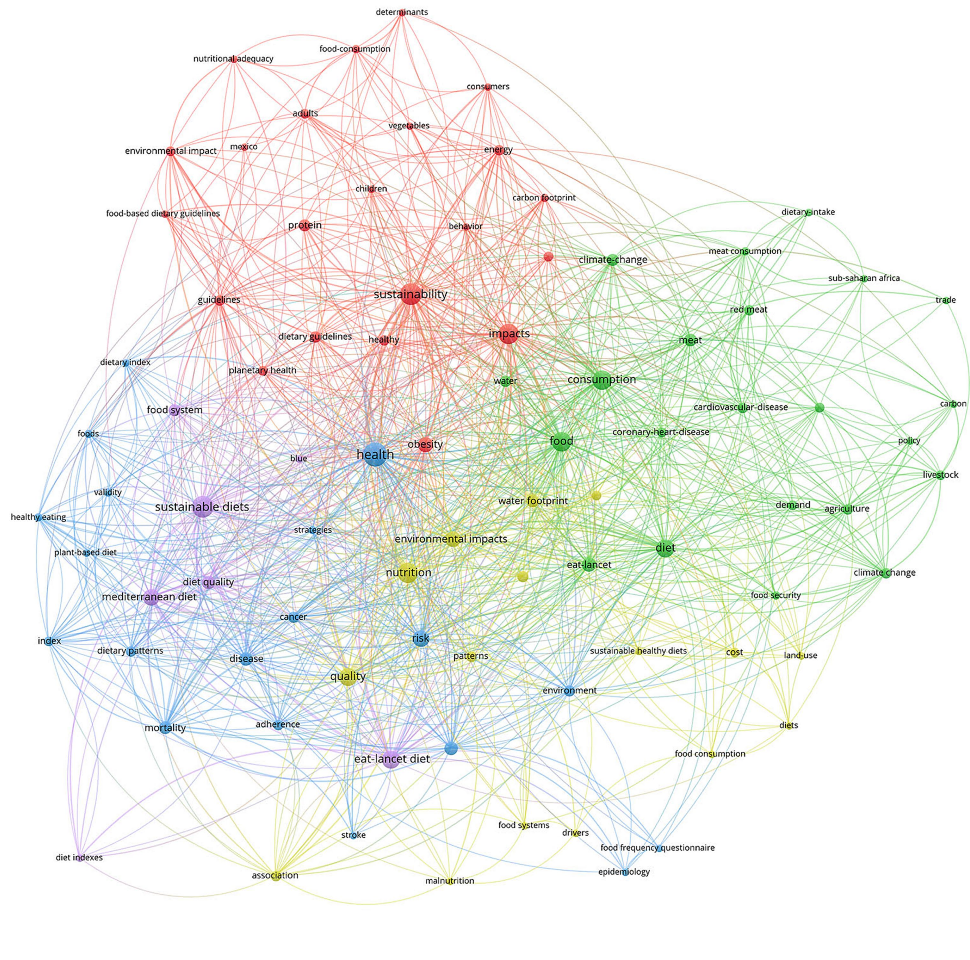

FIGURE 6
Visualization of keyword co-occurrence analysis.

researchers believed that that the diet might not provide adequate amounts of certain nutrients sourced mainly from animal products, such as vitamin B12, iron, calcium, and omega-3 fatty acids. Though these concerns can be addressed with fortified foods and supplements, it remains a point of contention.

The benefits of EAT-Lancet diet for human health

The EAT-Lancet diet has emerged as a global benchmark for optimal health, addressing critical health conditions while also championing environmental sustainability through lower carbon emissions and prudent resource use. Its impact has been the subject of various research efforts, particularly concerning diseases like diabetes, cardiovascular diseases (CVDs), and cancer (51–58). For instance, an insightful study delved into how closely following the EAT-Lancet diet influenced the occurrence and death rates from lung cancer (54). This research, based on data from the Prostate, Lung, Colorectal, and Ovarian (PLCO) trial involving 101,755 American adults, sought to clarify previously ambiguous connections between the diet and lung cancer. The findings were compelling, revealing that strict adherence to the EAT-Lancet diet corresponded to a substantial reduction in both the development and fatality rates of lung cancer, especially prevalent in cases of non-small-cell lung cancer. This suggests that maintaining the EAT-Lancet diet could potentially lower lung cancer risks. Colizzi and colleagues (59) focused on creating a diet score reflecting the Healthy Reference Diet (HRD) and evaluating its correlation with cardiovascular outcomes and ecological repercussions, drawing on data from 35,496 individuals in the EPIC-NL study. The results indicated that participants who closely followed the HRD registered lower probabilities of experiencing cardiovascular disease (14% reduction), coronary heart disease (12% reduction), and stroke (11% reduction). Another research initiative involving 24,494 participants investigated the link between the largely plant-focused EAT-Lancet diet (58), known for its dual benefits on human health and ecological sustainability, and the likelihood of T2DM. Following a median 24.3-year observation period, the study noted a striking 18% risk reduction for T2DM among those most committed to the EAT-Lancet diet compared to their least compliant counterparts. Interestingly, the diet's effectiveness seemed unhindered by genetic factors predisposing individuals to T2DM, suggesting its protective role regardless of genetic liabilities. One such research effort focused on understanding the broader health repercussions of the globally advocated EAT-Lancet diet led to the formulation of a novel dietary index to measure compliance. This study, utilizing the Malmö Diet and Cancer cohort's data, comprising 22,421 Swedes aged 45–73 years, examined dietary habits over roughly two decades

(56). The conclusions were significant: high fidelity to the EAT-Lancet diet was associated with a 25% decline in the risk of death from all causes, with remarkable reductions concerning cancer and cardiovascular-related deaths. These findings highlight the extensive health advantages of the EAT-Lancet diet, bolstering the case for its integration into sustainable eating protocols worldwide. Moreover, certain studies have indicated that higher adherence to the EAT-Lancet diet correlates with decreased overall mortality risks (1, 60).

However, the EAT-Lancet diet has sparked significant debate and criticism. Critics argue that its one-size-fits-all approach overlooks cultural and individual dietary needs, potentially leading to nutritional deficiencies in populations accustomed to different eating habits (61, 62). Beal et al. (5) evaluated the estimated micronutrient shortfalls of the EAT–Lancet diet, and found that the EAT–Lancet diet might not provide adequate nutrients, especially in micronutrients like iron, calcium, and zinc, potentially leading to some public health issues. This diet, focusing on minimally processed plant foods and low in animal sources, may require adjustments, such as increasing nutrient-dense foods like fish, shellfish, seeds, eggs, and beef, and decreasing foods high in phytate. Achieving dietary nutrient adequacy sustainably for the global population involves complex trade-offs between environmental preservation, reducing non-communicable diseases (NCDs), and nutrient adequacy. This calls for further analysis using dietary optimization modelling and life cycle assessments. The study suggests moving away from a one-size-fits-all planetary health diet towards context-specific guidelines, considering local data, cultural contexts, and environmental conditions. It emphasizes the need for inclusive approaches involving all stakeholders and underscores the integral link between human health and environmental preservation in addressing these global challenges. In addition, concerns have also been raised about the feasibility of its widespread adoption, given the varying agricultural capabilities and economic conditions across regions. Hirvonen et al. (21) investigated the financial feasibility of the EAT–Lancet diet. Analyzing food price and income data from 159 countries, the study found that the most affordable EAT–Lancet diets averaged $2.84 per day in 2011, with fruits and vegetables being the most expensive component. While affordable in high-income countries, this diet is beyond the financial reach of the world's poor, with an estimated 1.58 billion people unable to afford it. The cost of the EAT–Lancet diet also exceeds that of a minimum-cost diet meeting essential nutrient requirements by about 60% on average. This indicates that adopting the EAT–Lancet diet widely would require a combination of higher incomes, nutritional assistance, and lower food prices, alongside both local and systemic interventions to reduce the cost of healthier foods. Additionally, there's skepticism about the diet's impact on global food systems, with some suggesting it could exacerbate food insecurity in less affluent areas. Proponents, however, emphasize its potential benefits for health and the environment, advocating it as a necessary shift in the face of climate change and a growing global population. Despite these controversies, the EAT-Lancet diet continues to influence discussions on sustainable diets and global health.

There were some limitations in our study. We utilized the WoSCC database, and other databases like Embase and Pubmed are not used, due to the VOSviewer software's inability to process, analyze, and visually represent co-citation maps from their data. The total volume of publications concerning the EAT-Lancet diet is comparatively limited, indicating an immediate need for additional research. Expanding our understanding of the EAT-Lancet diet's impact on human health is critical, as is leveraging this dietary approach for health enhancement purposes.

In conclusion, our study is the first bibliometric analysis of EAT-Lancet diet. The main research hotspots and frontiers are the adaptation of EAT-Lancet diet, the composition of EAT-Lancet diet, and the benefits of EAT-Lancet diet for human health. The number of research on the EAT-Lancet diet is currently limited. There is a pressing need for further studies to broaden our understanding of the EAT-Lancet diet and its potential to enhance human health.

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 authors.

Author contributions

XL: Data curation, Formal analysis, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. SW: Data curation, Formal analysis, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. YG: Formal analysis, Funding acquisition, Project administration, Resources, 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.

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Analysis of the nutritional composition and organization of school meals in the province of Kadiogo in Burkina Faso: challenges and prospects

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Background: In the face of food shortages and precariousness, school meals are an effective means of encouraging pupils to attend and stay in school, and of combating nutritional deficiencies. Unfortunately, there are bottlenecks to be identified and resolved.

Objective: Analyzing the composition of meals served to school-age children in primary schools in the province of Kadiogo, while assessing the opinion of school staff on these meals (Burkina Faso).

Methods: A descriptive cross-sectional survey about school meals was carried out during the period from April to May 2019 among school stakeholders in primary schools in five (05) municipalities of the province of Kadiogo.

Results: Insufficient quantity and quality of rations served were recorded in primary schools. The endogenous initiative canteens represented 46.4% of the registered canteens. The promotion of Health-Hygiene-Nutrition (H-H-N) activities in schools encountered difficulties in covering the sanitary needs of school-aged children because unavailability of socio-sanitary infrastructures. School meals consisted of starchy foods and legumes in rural schools and more diversified meals consisting of fruits and vegetables as well as meat and fish in urban schools. In rural municipalities, school meals were insufficient in quantity and quality, while in the urban municipality, macronutrient intakes were in excess with micronutrient intakes largely deficient.

Conclusion: Despite the shortcomings, school officials specified that school meals cover lunch rations, increase school enrolment, and improve school-aged children' learning capacity.

KEYWORDS

school meals, canteen, food ration, school-aged children, micronutrients, macronutrients

1 Introduction

Like many countries in sub-Saharan Africa, Burkina Faso has begun a demographic transition with an average annual growth rate of 3.1 and 48% of the population is under 15 years of age (1). Although decreasing, the mortality rate is still high, in part, because of the double burden of malnutrition, which mainly affects school-age children. Given the importance of healthy nutrition for young children and its implication for their contribution to societal development, it is important to have nutrition-specific interventions targeting young people (2–4). Such interventions will enable countries in transition, such as Burkina Faso, to benefit in the future from healthy human resources capable of driving sustainable development. Therefore, the challenge is how to make such investments a priority in the basic social sectors including education and health (5). It is in this context that school feeding programs called school canteens are established. These programs consist of serving meals to pupils or giving them food rations to take home. These school canteens operate through institutional school feeding programs implemented by different agencies depending on the region (6). The agencies' contributions are often complemented by the participation of communities and families, hence the name "endogenous canteens." The initial objective of school canteens was to make them effective support tools for the improvement of school results and thus to positively impact the quality and performance of the education system as a whole (7). However, in the face of food insufficiency and precariousness, these school meals have very quickly established themselves as effective means of promoting access to and retention in school, but also as a means of developing education and combating nutritional deficiencies (8). School canteens have been in existence for many years and their effect as an enhancer of children's school attendance and performance has been demonstrated in several countries. However, the importance of canteens in the diet and nutritional status of children remains poorly documented.

Many challenges remain, particularly in terms of organization and mobilization of financial and logistic resources for the optimization of programs. In this regard, one of the bottlenecks is the inability to provide school-aged children with meals sufficient in quantity and quality on a permanent basis throughout the school year given the national scope of the program. In this context, the present study aimed at analyzing the meals served to school-aged children in primary school in the province of Kadiogo in Burkina Faso.

2 Methodology

2.1 Study site

This study was conducted in primary and private schools with school canteens in the province of Kadiogo, specifically in the rural communities of Komki-Ipala, Tanghin Dassouri, Pabré, Saaba, and urban communitie of Ouagadougou (Figure 1).

2.2 Study type, period, and population

This is a descriptive cross-sectional study. The study took place over a two-month period, from April to May (2019). The study population was responsible for primary schools. Data collection consisted of recording school officials' assessments of school meals

FIGURE 1
Representative map of the Kadiogo province.

and analyzing the composition of meals served in public and private primary school canteens selected in Kadiogo province.

2.3 Sampling and data collection

The sampling of schools for each municipality was done in Open-Epi. Primary schools were selected on the basis of the availability of a school canteen and in a reasoned manner of school/municipality from all primary schools in the province of Kadiogo. Five municipalities were selected out of the seven municipalities (9) of the province including five schools per municipality (three public schools, two private) schools in the four rural municipalities and 11 schools in the urban municipality (10).

2.4 Inclusion criteria

All public and private educational schools with school canteens were considered during the sampling. These canteens must operate on a regular basis and be part of the selection criteria in the province of Kadiogo. Excluded from this study were educational schools that had already exhausted their foodstuff and school officials who refused to participate in the study.

2.5 School meals composition analysis method

The food group composition of school meals was analyzed, using the list of food groups suitable for school-aged children as well as food composition tables (11). The nutritional value of the different meals was calculated taking into account the amount of ingredients, the method of preparation and the yield and nutrients retention factors according to Bognar description (12).

2.6 Data processing and analysis

The analysis of the data on the school official' evaluation of school meals was carried out using SPSS version 20.0 software and an appropriate input mask developed for this purpose. The Chi-square test was used to compare the variables. For all tests and comparisons, the significance level was set at $p \leq 5\%$.

3 Results

Of 31 schools identified for the study, 28 school officials were interviewed. Schools interviewed included 21 public and 7 private schools. The three schools excluded from the study were schools whose canteens had exhausted their food supplies. Approximately 46.4% of the educational structures surveyed had installed endogenous canteens within their establishment.

3.1 Report from school officials

The survey of school officials shows that they recognize six objectives for school meals, of which the objectives of improving academic performance and increasing children's attendance at school ranked first and second (Figure 2).

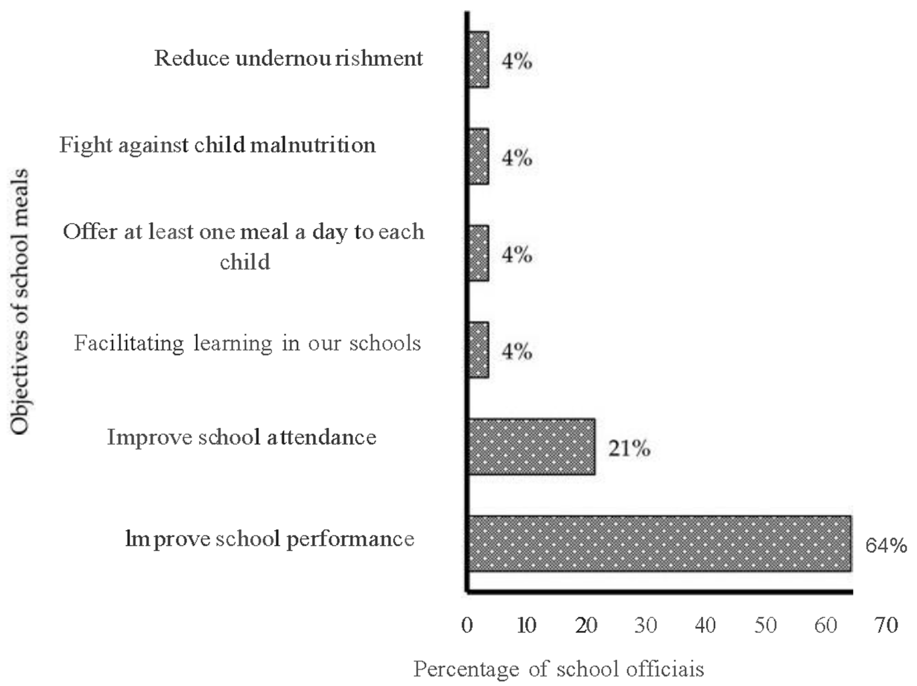

FIGURE 2
Goals of school meals according to school officials.

TABLE 1 Assessment of canteens by school officials.

Estimation of the quantity of food received	n	%
Sufficient	2	7.1
Average	8	28.6
Insufficient	18	64.3
Total	28	100.0

TABLE 2 Promotion of Health-Hygiene-Nutrition activities in schools.

Promotion of HHN activities in schools	YES		NO	
	N	%	N	%
Deworming of school-aged children	8	28.6	20	71.4
Supplementation with vitamine A	4	14.3	24	85.7
Iron supplementation	0	0.0	28	100.0
Iodine supplementation	2	7.1	26	92.9
Medical kit	8	28.6	20	71.4
Sanitation activities	28	100.0	0	0.0
Sensitization on HHN	11	39.3	17	60.7
Participation in competitions on HHN	7	25.0	21	75.0

Regarding food supplies, 64% of officials surveyed reported that the amount of food provided was insufficient to cover school-aged children' food needs throughout the school year whereas, 29% mentioned that the quantities were average and 7% of them reported that the quantity was sufficient (Table 1).

3.2 Appreciation of canteens regarding the quality of food received

The study found that 64.3% of school canteen cooks rated the physical quality of received food as average and 35.7% of cooks found the food to be of poor quality.

3.3 Promotion of health-hygiene-nutrition activities in schools

Only 28.6% of schools had received deworming products for their school-aged children and the majority of schools (71. 4%) had not benefited from it. However, the goal is to combat intestinal worms and some skin diseases in children. With regard to school-based supplementation activities, only 14.3% had been supplemented with vitamin A, and 7.1% with iodine. No school received iron supplementation. It can be established that the majority of school-aged children were not receiving micronutrient supplementation. Twenty-eight point 6 % (28.6%) of educational facilities had a first-aid medical kit and 39.3% were able to sensitize the community on health, hygiene and nutrition activities (Table 2).

3.4 Composition of school meals

According to the school meals composition by food groups, 9 food groups defined by the FAO (13) for school-age children, only 4 were included in the meals served, namely starchy foods; legumes, nuts and seeds; fruits and vegetables; and meat and fish (Table 3). This was mostly true in the urban municipality. In rural areas, the majority of school-aged children in public schools consumed only 2 groups (cereals and legumes) out of the 9 with the exception of a few private schools that went beyond 03 food groups (adding cereals, legumes, or fish).

3.5 Benefits of school meals for education

85.7% of school officials believe that school meals increased school enrolment and strengthened the relationship with parents. Also 35.7% of the school officials had seen the reality of the endogenous canteen within their schools and 3.6% of the schools had set up a school field which is a garden set up in the schools to improve the provision of balanced meals (Table 4).

3.6 Comparison of who recommended nutritional requirements to the composition of school meals

During the surveys in the selected schools of the Kadiogo province, only one lunch ration was recorded per day, which was cereal base. Using the nutrient contents per 100 g of school meals, the nutrient contents of the pupils' lunch ration were calculated according to the recommended scales (150 g of rice, 35 g of beans/mung beans and 16 mL of oil). These nutrient contents of the pupils' lunch ration were used to estimate daily intakes based on the WHO recommended nutrient requirements. Based on the above results, the energy intake of some rations was above the recommended value. Meals such as Soumbala rice, Jollof rice, and Mung Beans rice contributed 165.8, 161.7, and 182.7% of energy requirements. These types of meals were seen in the urban community with excess energy intakes.

TABLE 3 Composition of school meals.

Food group covered by school meals	YES		NO	
	Consumed		Not-consumed	
	n	%	*n*	%
Fruits and vegetables	12	42.9	16	57.1
Meat and fish	12	42.9	16	57.1
Legumes, nuts and seeds	28	100.0	0	0.0
Milk and milk products	0	0.0	28	100.0
Starchy food	28	100.0	0	0.0
Other Fruits and vegetables rich in vitamin A	0	0.0	28	100.0
Eggs	0	0.0	28	100.0
Offal	0	0.0	28	100.0
Dark green leafy vegetables	0	0.0	28	100.0

TABLE 4 Benefits of school meals in schools.

Benefits of school meals for education	YES		NO	
	n	%	*n*	%
Improves school enrollment rate	24	85.7	4	14.3
Improves academic performance	28	100.0	0	0.0
Decreases the number of grade repeats	28	100.0	0	0.0
Decreases the number of dropouts	27	96.4	1	3.6
Improves school completion rate	28	100.0	0	0.0
Improves academic results	28	100.0	0	0.0
Improves nutritional status	28	100.0	0	0.0
Promotion of endogenous canteens	10	35.7	18	64.3
Establishment of school gardens	1	3.6	27	96.5
Strengthens relationships with parents	24	85.7	4	14.3
Ensures the maintenance of children	28	100.0	0	0.0

The data also showed that protein intakes are relatively low and lipid intakes were high for all municipalities surveyed. On the other hand, the carbohydrate intakes of Jollof rice (120.2%), soumbala (fermented nera grain) rice (120.9%) and mung beans rice (127.7%) far exceeds the requirement. As a result, the school meals' content in macronutrient (especially carbohydrates) and energy requirements was above the requirements in the urban municipality. Except for mung beans rice, which contributes 21.7% of phosphorus requirements, and Jollof rice, which contributes 36% of vitamin A requirements, no other type of school meal contributed sufficiently to the micronutrient requirements for those assessed (Table 5).

4 Discussion

School officials in this study noted that through the implementation of school canteens, school-aged children' academic performance has improved significantly. The enrolment rate of children has increased considerably since the introduction of the school feeding program in public and private schools. School meals would also be a means of combating malnutrition among school-aged children. The objectives of school meals cited by principals also contribute to the achievement of the objectives set by WHO (14) for school meals, which included improving school enrolment, attendance and performance WHO (14).

The quantity of food received in the schools surveyed was insufficient because the allocation lasted only three months out of nine (09) during the entire school year. These shortcomings are often made up for by the presence of an endogenous canteen in the schools. The adequacy of school meals in schools was conditioned by the interventions of certain non-governmental organisations (NGOs). In any case, the operation of the canteens is part of the administrative framework of Burkina Faso education system, which is decentralized at all territorial levels (15). The government's decision to transfer the management of school canteens to the communities contributes to the strengthening of the implementation of full communalization and the responsibility of communities for economic development at the grassroots level.

To solve this problem of inadequacy, opinion leaders in the municipalities have developed initiatives at the local level to mobilize resources for the permanent operation of school canteens within the various schools. Also, the irregularity of the allocations was one of the

TABLE 5 Comparison of WHO nutritional requirements to the composition of school meals.

Meal		Couscous beans	Soumbala rice	Jollof rice	Mung beans rice	Rice-beans	Nutritional requirements
Energy	(Kcal)	260.8	707.9	690.6	780.1	260.8	427.0
	%	61.1	165.8	161.7	182.7	61.1	
Protein	(g)	7.9	13	10.8	17.9	7.9	41.0
	%	19.3	31.7	26.3	43.7	19.3	
Lipids	(g)	18.1	19.3	18.4	18.7	18.1	21.3
	%	85	90.6	86.4	87.8	85	
Carbohydrates	(g)	20.2	120.9	120.2	127.7	20.2	100.0
	%	20.2	120.9	120.2	127.7	20.2	
Calcium	(mg)	30	59.7	61.5	40.3	30.0	800.0
	%	3.8	7.5	7.7	5.0	3.8	
Magnesium	(mg)	0.0	0.0	0.0	49.4	0.0	130.0
	%	0.0	0.0	0.0	38	0.0	
Iron	(mg)	2.3	3.8	3.0	4.6	2.3	17.8
	%	12.9	21.3	16.9	25.8	12.9	
Phosphorus	(mg)	0.0	0.0	0.0	108.6	0.0	500.0
	%	0.0	0.0	0.0	21.7	0.0	
Vitamin A	(μg E.R)	4.2	0.0	143.9	0	4.2	400.0
	%	1.1	0.0	36.0	0	1.1	
Vitamin C	(mg)	0.2	0.0	1.5	0.6	0.2	25.0
	%	0.8	0.0	6.0	2.4	0.8	

reasons for the organization of the endogenous canteens. To remedy this, it will be necessary to provide schools with food at the beginning of the year in order to improve the implementation of endogenous canteens. OXFAM's 2015 report stated that irregular allocations and delays in delivery were holding back the program. The complexity of the logistics required to supply all schools in the country on time, particularly in the state-run provinces, the magnitude of the program and the limited resources allocated to it leads to dysfunctions resulting in delays in deliveries. This makes it difficult to organize and plan school canteens and establish endogenous canteens (16).

The quantity was insufficient, but the quality was also lacking, and this was explained by the fact that the food was already damaged on delivery, especially the beans whose quality left something to be desired despite the checks carried out before delivery. A study on social protection and food security in Burkina Faso in an Oxfam report in 2015 indicated that 54% of the surveyed canteens had judged the products distributed in school canteens to be of poor quality (16).

The promotion of Health, hygiene, and nutrition (H-H-N) actions and their implementation encountered difficulties in their implementation because of the lack of support and the actions that were implemented were not sufficient to cover the health needs of school-aged children because of the lack of availability of some socio-sanitary infrastructures in the communities.

In the selected public education facilities in Kadiogo province, only one ration (rice-beans) was documented per day at lunch, which consisted of a cereal base. And this ration contributes to only 61.1% of the school-aged children daily nutritional energy requirements based on daily intakes to ensure normal nutrition in a healthy school-aged children (17). According to FAO, a daily consumption of two food groups could not cover the dietary needs of school-aged children especially in micronutrients while for a number of school-aged children especially in rural areas, school meals should be a privileged source of nutrients essential for their growth and psychomotor development. In most cases, the dietary diversification of school meals remains a challenge to be met in all municipalities of Kadiogo. In rural communities, school meals are insufficient in quantity and quality; micronutrient intakes were largely deficient. In view of the above results, vitamin A supplementation is necessary unless household consumption can cover this deficit because micronutrient deficiencies have profound effects on the health of the brain, bones and the body in general.

The presence of school meals contributed to improved academic performance, retention of school-aged children in school, completion rates and reduced dropouts. In 2013, in Ghana, a study on the school feeding program corroborated the benefits cited above by the principals of the schools surveyed. The implementation of this program has increased enrolment in primary schools in Ghana (18).

5 Conclusion

This study highlighted the realities of school nutrition in Burkina Faso. The study showed that school meals were about the same, neither balanced nor varied in most primary schools and that the coverage of school-aged children' micronutrient nutritional needs was deficient. Despite the realities of school feeding, the results of the study show that school meals play an important role in education as they reduce hunger, drop-outs, repetition rates, increase enrolment, attendance, academic performance, improve attention, school-aged children' learning capacity and school completion rates. School meals made it possible to provide at least one meal a day. In view of the results, we suggest that further descriptive cross-sectional studies be conducted to better assess school-aged children' diets at the household and school levels in order to assess their daily consumption in general.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number (s) can be found below: the database used in this study can be provided by the corresponding author upon reasoned request.

Ethic statement

The study required the consent of the inspectors of the municipalities, the Directors of primary schools, rural and urban communities as well as the managers of school canteens and the cooks.

Author contributions

EC: Conceptualization, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. OO: Conceptualization, Formal analysis, Investigation, Methodology, Software, Supervision, Writing – original draft, Writing – review & editing. TS: Writing – original draft, Writing – review & editing. MB: Writing – original draft, Writing – review & editing. MS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Writing – original draft, Writing – review & editing. MD: Funding acquisition, Writing – original draft, Writing – review & editing.

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The importance of taste on swallowing function

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The world's population is aging. Pneumonia is the leading cause of death among the older adults, with aspiration pneumonia being particularly common. Aspiration pneumonia is caused by a decline in swallowing function. Causes can include age-related sarcopenia of swallowing muscles, cognitive decline, cerebrovascular and other diseases or even changes in individual taste preference. Currently, the main treatment approach for dysphagia is resistance training of swallowing-related muscles. This approach has not been effective and establishment of novel methods are required. In this review, we introduce and discuss the relationship between taste, taste preference, carbonation and swallowing function. Taste and preference improve swallowing function. Recently, it has been shown that a carbonated beverage that combines the functionality of a thickening agent, the appeal of taste, and the stimulation of carbonation improves swallowing function. This may be very useful in the recovery of swallowing function. It is important to note that deliciousness is based not only on taste and preference, but also on visual information such as food form. Umami taste receptors are expressed not only in taste buds but also in skeletal muscle and small intestine. These receptors may be involved in homeostasis of the amino acid metabolic network, i.e., the process of amino acid ingestion, intestine absorption, and storage in skeletal muscle. Proper stimulation of umami receptors in organs other than taste buds may help maintain nutritional status and muscle mass. Umami receptors are therefore a potential therapeutic target for dysphagia.

KEYWORDS

aspiration pneumonia, dysphagia, carbonated thickened drink, taste receptors, skeletal muscle, taste, preferences

1 Introduction

Throughout life, the ability to eat well and savor a meal remains one of the most important factors in pursuing happiness. Therefore, developing proper swallowing functions in childhood and maintaining them throughout life is essential.

In 2017, 1.13 million people over the age of 70 (261/100,000) died from secondary community-acquired pneumonia (CAP), a 9% increase in that mortality rate over the past 30 years (1). CAP in the elderly population frequently stems from aspiration pneumonia (AP). Aspiration pneumonia has been proposed as a physiologic phenomenon caused by aspiration due to the age-related decline in swallowing function and cough reflex (2). Furthermore, stroke and prolonged bed rest have been identified as risk factors for aspiration. However, there are still no uniform global diagnostic criteria for aspiration pneumonia. Certain cultures regard and treat aspiration pneumonia in cases of obvious aspiration associated with head and neck cancer or stroke. However, those associated with senility are not considered for active intervention. The proportion of aspiration pneumonia among pneumonia cases that result in hospitalization increases with age, and non-aspiration pneumonia is less common among older adults (3, 4). In other words, aspiration pneumonia poses a significant challenge that must be addressed to ensure healthy longevity in today's aging society worldwide.

In this review, we would like to discuss the role of taste sensation in swallowing motor functions, their disorders, and the functions of taste receptors expressed outside the oral mucosa. We can examine strategies for overcoming swallowing disorders, paving the way for a healthy and fulfilling life throughout our entire lifespan.

2 Population aging worldwide and dysphagia/aspiration pneumonia

Swallowing disorders are characterized and classified by the following: [1] Inability to form a bolus of food in the oral cavity and to transport the bolus to the pharynx. [2] Misdirection of food and fluid in the pharynx, causing it to enter the trachea instead of the esophagus. [3] Inability to get rid of food or liquid accidentally entering the trachea by coughing (5).

In today's aging society, aspiration pneumonia due to dysphagia in older adults is becoming an increasing problem (6). Older adults over 65 years old account for two-thirds of those affected by dysphagia (7). In the United States, aspiration pneumonia was reported as the underlying cause of death in an average of 17,616 cases per year, representing 30.1% of all aspiration pneumonia-related deaths. Individuals aged 75 and over accounted for 76.0% of deaths from aspiration pneumonia, with an age-adjusted rate ratio of 161.0 (CI 160.5–161.5) (8). Maintaining oral hygiene and swallowing function is important in preventing aspiration pneumonia (9).

3 Swallowing and skeletal muscle

Various muscles are involved in swallowing movements. They include the masticatory muscles, the tongue, and the perifacial muscles involved in mastication movements. They include the supralaryngeal muscles involved in elevating the larynx. The sublingual muscles support the hyoid bone, and the pharyngeal contractile muscles increase swallowing pressure. However, this is not the only cause of dysphagia, as poor posture with aging, such as hunchback and neck anteflexion, can lead to compensatory use of muscles needed for swallowing, resulting in secondary dysphagia (10).

Although sarcopenia is a disease characterized by generalized muscle loss, muscle weakness, and loss of physical function, the muscles associated with swallowing do not seem to lose muscle mass and strength like other muscles. These muscles, except the geniohyoid muscle, are actively engaged in respiration, even at rest, under the control of the respiratory center. Therefore, it has been thought that disuse muscle atrophy is unlikely to occur in the muscles associated with swallowing (11). In contrast, there are reports of accelerated muscle atrophy of the tongue and diaphragm in patients with aspiration pneumonia (12), and studies show that muscle atrophy occurs in the respiratory muscles and muscles associated with swallowing (13).

Various types of training are available to improve swallowing function, including resistance training of swallowing-related muscles (14–16) and neuroelectrical stimulation therapy (17).

4 Taste receptors and skeletal muscle

The Tas1R family (Tas1r1, Tas1r2, and Tas1r3) are G protein-coupled receptors that sense sweet taste in the Tas1r2/Tas1r3 complex and umami taste in the Tas1r1/Tas1r3 complex as taste receptors. Recent studies have revealed that the Tas1R family members function as nutrient sensors in tissues other than the oral mucosa (18).

Skeletal muscle is one of the largest organs in the human body, accounting for approximately 40% of body mass. With age, skeletal muscle mass tends to decrease, leading to a condition known as sarcopenia. Sarcopenia is characterized by muscle weakness and decreased physical activity capacity, which can significantly impact the quality of life for older adults. Since muscle is a central organ that takes up and consumes sugar and fatty acids from the blood, its maintenance is considered essential for preventing metabolic-related diseases such as obesity and diabetes (19).

Satellite cells are muscle stem cells that provide a regenerative capacity to skeletal muscle. Aged skeletal muscles have an impaired regenerative capacity which can contribute to physical incapacitation. Aged skeletal muscles fail to retain stem cell quiescence (20–22). Muscle stem cell number and the functionality decline with aging (20–25). The process of autophagy, which involves the degradation of long-lived proteins and damaged organelles in lysosomes, has been implicated in the aging of different model organisms (22, 26–29). Maintenance of skeletal muscle mass also relies on the balance between anabolic and catabolic processes. Protein degradation in skeletal muscle cells is essentially mediated by the activity of two conserved pathways: the ubiquitin proteasomal pathway and the autophagic/lysosomal pathway (30). The ubiquitin-proteasomal pathway is responsible for the turnover of the majority of soluble and myofibrillar muscle proteins (31, 32). Autophagy also plays an important role in the degradation of skeletal muscle (33).

Tas1r1 and Tas1r3, are highly expressed in muscle relative to other tissues (34). We reported that myogenic regulatory factor (MRF)s regulate the expression of Tas1r3 (35). Overexpression of MyoD and Myogenin induces murine Tas1r3 promoter activity. ChIP analysis demonstrated that MyoD and Myogenin bind to the endogenous murine Tas1r3 promoter and increase mRNA levels of endogenous Tas1r3 in murine myoblasts. We demonstrated that the expression of Tas1r1 also increased during myogenesis in a cell culture model (36). These findings are further supported by the observation that Tas1r1 and Tas1r3 are endogenously expressed in skeletal muscle tissue. The skeletal muscle Tas1r3 knockout mice exhibit decreased activity of the mammalian target of rapamycin complex 1 (mTORC1) and a higher frequency of autophagy (34), suggesting that umami receptor function is critical to detecting nutrient status since skeletal muscle is the main source of stored amino acid during times of amino acid deprivation (37). For these reason, we hypothesized that disorder of signaling through Umami receptor is involved in pathogenesis of skeletal muscle related diseases including sarcopenia and swallowing disorders.

5 Rehabilitation, nutrition, and taste

Adequate nutrition is essential for enhancing the effectiveness of rehabilitation through strength training, and the concept of rehabilitation nutrition is gaining increasing recognition (38). The importance of the trinity of rehabilitation, nutrition, and oral management for sarcopenic dysphagia in older adults is emphasized (39). Taste is essential to sustain life, and its primary function is to facilitate the intake of essential nutrients and the rejection of harmful substances. Umami receptors are expressed on epithelial cells in the small intestine. Glutamate stimulation via umami receptors in the small intestine is essential for maintaining the normal turnover of small intestinal epithelial cells (40). Insufficient umami stimulation of umami receptors in the small intestine can lead to impaired nutrient absorption from the small intestine. To ensure optimal nutrient intake, it is necessary to study the sense of taste and the regulatory mechanism of nutrient absorption via taste receptors in the intestinal tract.

6 Swallowing function and sense of taste and preferences

Swallowing function is known to be influenced by individual preferences. High temporal resolution fMRI studies show that taste, smell, and visual sensations stimulated by highly palatable foods, such as popcorn and chocolate, activate areas of the cerebral cortex associated with swallowing (sensorimotor area, insular cortex, cingulate gyrus, prefrontal cortex) (41). Taste stimuli alter the swallowing reflex. Taste, particularly acidity, increases swallowing pressure and suprahyoid muscle activity (42–44). Other reports indicate that salty and sweet stimuli increase swallowing pressure and suprahyoid muscle activity (43–45). However, foods with intense sour or salty flavors that directly trigger swallowing are considered problematic regarding taste and palatability (43). Conversely, there is a positive correlation between taste and swallowing function. Salty and sweet stimuli are more effective than no-taste stimuli in increasing swallowing pressure and supratrochlear muscle activity (42, 44, 45). A study involving young, healthy subjects compared the ease of swallowing foods with the five basic tastes (sweet, salty, sour, bitter, and umami) against tasteless foods. Sweet and tasteless foods exhibited slightly better swallowing acceptability than by sour and bitter foods (46). Investigations involving healthy adult male volunteers have shown that cortical swallowing pathways are similarly modulated by both sweet and bitter stimuli (46). This study implies the existence of a close interaction between taste perception and swallowing activity via the central nervous system.

7 Swallowing function-carbonation and taste

Swallowing movements are not merely simple muscle contractions; rather, they are controlled by various nerves. Stimulating these sensory nerves is important for maintaining their functionality and overcoming swallowing dysfunction. Using carbonated beverages as a stimulatory modality has recently garnered attention for its efficacy in enhancing swallowing movements. Carbonated water increases muscle activity during swallowing more than plain water (47), and the higher gas volume of carbonated water increases tongue pressure (48). In addition to its effect on muscle activity, carbonated water improves pharyngeal clearance with significantly faster pharyngeal transit time compared to normal liquids (49). Furthermore, carbonated water supports the shortening of tablet transit time during tablet swallowing (50). Furthermore, carbonated water is less likely to be aspirated or left in the pharynx than non-carbonated liquids in patients with central nervous system disease (51, 52). We and other laboratories have reported that thickened carbonic acid further improves swallowing function (53, 54).

As one of the reasons why carbonated beverages improve swallowing, H_2CO_3 produced by carbonic anhydrase IV in saliva from CO_2 dissolved in carbonated beverages stimulates nociceptors in the oral mucosa and activates the trigeminal nerve (55). In addition, the carbonic acid in carbonated water splits into bicarbonate and hydrogen ions, and the hydrogen ions stimulate the facial nerve via acid-sensitive taste receptors (56). Stimulation with capsaicin improves swallowing function (57).

Capsaicin-induced improvement of swallowing involves TRPV1 at the nerve terminal. ASIC3 is a receptor expressed at nerve endings similar to TRPV1. The ASIC3 receptor has been implicated in the effects of carbonic acid stimulation on swallowing (58).

In contrast, there are reports that swallowing only carbonated water can worsen the penetration-aspiration scale (PAS) and videofluoroscopic dysphagia scale (VDS) (59, 60). This is thought to be influenced by preference and taste (Please see preceding paragraph).

8 Swallowing and food forms

The available forms of food intake are frequently restricted due to poor swallowing functions. However, even if dysphagia-adjusted diets, such as kizami or paste diets, are suitable for the remaining swallowing functions, these dysphagia-adjusted diets do not maintain the original food form and may decrease the patient's desire to eat (61). Sight and smell control appetite (62). Other laboratories reported that the arrangement of coloring, smell, and flavor could increase food intake and improve nutritional status, while dysphagia-adjusted diets serve as food form (63–65). Eating well brings a sense of spiritual richness and satisfaction, and it helps establish and maintain social relationships and communication. Because social activities are often limited for older adults, meals are a major recreation component.

Yoshihara et al. (66) reported a positive correlation between appetite and quality of life in a study of an elderly community in Japan. Eating food that is not tasty solely for sustenance can be a disagreeable experience. Meals should be enjoyable to consume in terms of flavor and presentation. Older individuals exhibit different eating patterns compared to young children just starting to learn regarding food. Older individuals have had the pleasure of enjoying many delicious meals throughout their lives. The purpose of a meal extends beyond simply fulfilling nutritional and energy needs. The European Pressure Ulcer Advisory Panel's guidelines for pressure ulcer prevention and treatment include considerations for appetizing food with attractive presentations (67).

9 Discussion

Thus, swallowing function is closely related not only to mastication and pharyngeal muscle contraction but also to various stimulus inputs (Figure 1). Therefore, simple muscle training may not be sufficient to treat dysphagia. This review focused on swallowing functions regarding taste and taste receptors. Maintaining swallowing functions and taking adequate nutrients are necessary to maintain swallowing functions. On the contrary, poor nutritional status naturally leads to a decline in swallowing function. This is indeed a chicken and egg situation between "swallowing" and "nutrition."

Amino acids (proteins) in food are received by umami receptors (Tas1r1/Tas1r3) in the taste buds of the oral mucosa. These amino acids (proteins) are recognized as "delicious" taste sensations and are actively absorbed after digestion and stored in skeletal muscles as myofiber proteins. Muscle fibers are depleted during starvation, and amino acids are released into the bloodstream (37). In sarcopenia, this amino acid network (Figure 2) may be disrupted, leading to the breakdown of skeletal muscle proteins. This releases more amino acids that are taken up, causing a decrease in skeletal muscle mass and function. On the other hand, Tas1r3 knockout does not alter amino acid concentrations due to compensatory increases of other amino acid transporters (34). In addition to this, there are several issues that need to be resolved before deciding the function of taste receptor in skeletal muscle. For example, do Tas1r1 and Tas1r3 form a heteromeric complex in muscle cells similar to in taste bud cells? What is the ligand for taste receptors when they function as amino acid sensors in skeletal muscle? Careful *in vivo* experimental analysis focusing on skeletal muscle metabolism will be required to address these issues.

Humans with decreased sensitivity to umami taste were reported to show significant weight loss and deterioration in general health compared to those with other taste disorders (sweet, salty, sour, and

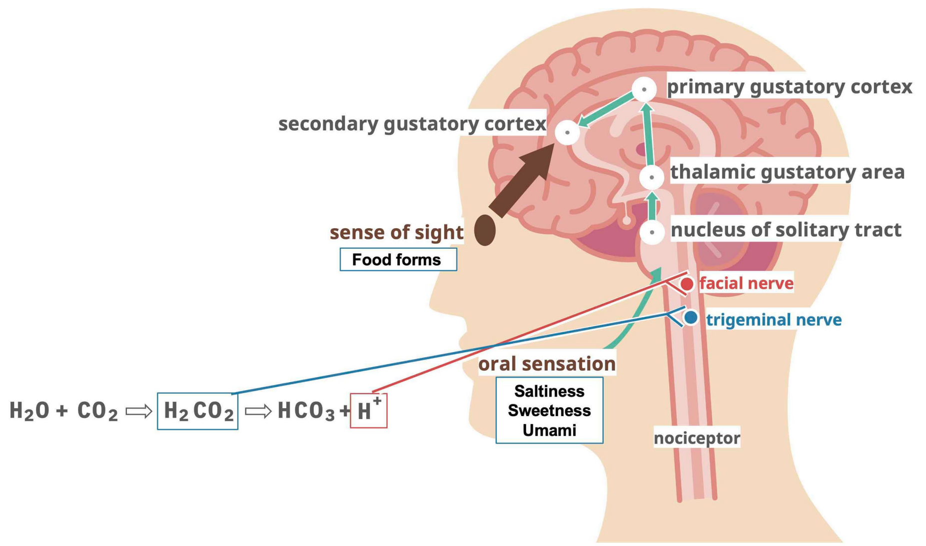

FIGURE 1
Hypothesized mechanisms of action for taste, vision, and carbonic acid stimulation.

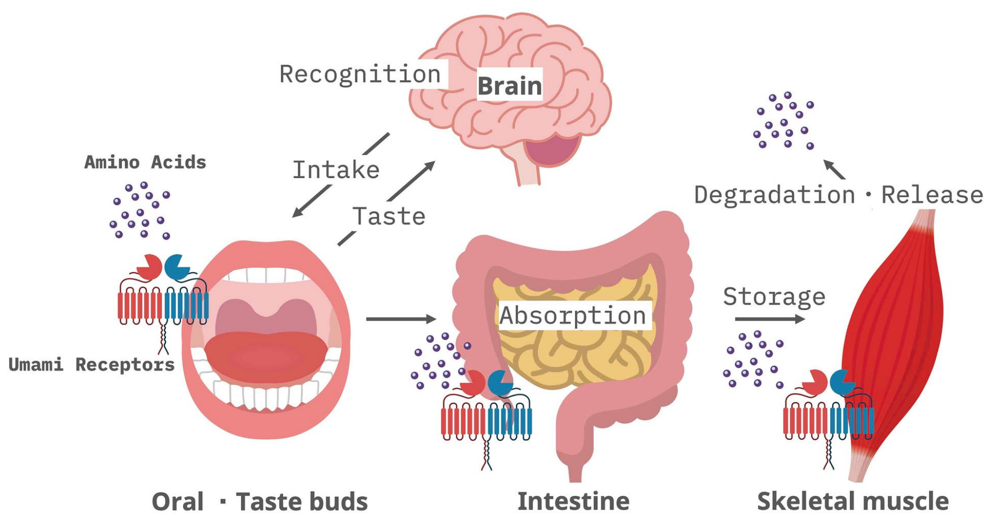

FIGURE 2
Amino acid network via umami receptors expressed by oral mucosa, intestine, and skeletal muscle.

bitter) (68). In this report, it was interpreted that the patients had difficulty perceiving umami, which may have made the food less palatable and reduced their food intake. Moreover, a reduction in the expression level and sensitivity of umami receptors at the genomic level diminishes taste perception and impairs the function of umami receptors in the small intestinal mucosa, skeletal muscle, and other organs that comprise the amino acid sensing network. In other words, the observed weight loss and decline in overall health may be manifestations of underlying nutritional deficiencies caused by impaired digestion and absorption and reduced skeletal muscle mass resulting from abnormal skeletal muscle metabolism. Multiple single nucleotide polymorphisms are observed in the gene encoding the umami receptor, with each mutation having a different sensitivity to the umami receptor *in vitro* (69). Therefore, based on their genetic background, humans have reduced sensitivity to umami receptors in the body.

Medical advancements have significantly extended our lifespans, allowing us to delay death's inevitable arrival. However, this progress has created a disparity between healthy life expectancy, the number of years an individual can expect to live independently in society, and overall life expectancy, which simply marks the end of life. In addition, the collapse of current healthcare systems and the economic disparity in the selection of life are becoming realities due to the extremely expensive drugs created by the latest life science and technology. If we can advocate an inexpensive extension of healthy life expectancy based on food, we can solve these problems and make a major shift toward a sustainable super-aging society.

This review article highlights the association between swallowing disorders and sarcopenia of the associated muscles. Diseases that cause skeletal muscle atrophy, such as sarcopenia, significantly reduce healthy life expectancy. Currently, no commercialized interventions, except for exercise, exist to maintain or restore skeletal muscle mass and function. Considering the high prevalence and pathogenesis of sarcopenia, the prevention and treatment of sarcopenia should be based on a healthy diet combined with adequate exercise rather than expensive medicine such as molecularly targeted drugs. Sufficient amino acid intake, a crucial component of skeletal muscle, is essential for preventing sarcopenia. Future research endeavors to identify amino acid combinations that efficiently activate the umami receptors of each cell in the amino acid network may contribute to overcoming sarcopenia and thereby extending healthy life expectancy in this super-aging society.

Author contributions

MO: Writing – original draft, Writing – review & editing. MM: Writing – review & editing. TO: Writing – review & editing. HS: Writing – review & editing. TK: Writing – review & editing. SK: Conceptualization, 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.

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Differences in time–intensity sensory profiles of sweet taste intensity of glucose between older and young adults

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Background: To understand age-related changes in sweet taste perception in daily life, it is important to understand taste intensity at the suprathreshold level. Previous studies have attempted to characterize the temporal aspects of human taste perception in terms of time–intensity evaluations. The perception of dynamic taste intensity in older adults increases slowly for salty taste; however, there have been no previous studies on time–intensity sensory evaluation of sweet taste in older adults. We hypothesized that older adults perceive sweet taste intensity more slowly than young adults.

Methods: Fifty young and 40 older adults participated in the study and glucose solutions of 0.6 M and 1.5 M were used as stimuli. The study comprised two experiments: (1) a cup tasting test (static taste perception in the mouth), and (2) a time–intensity sensory evaluation, in which the solutions were presented using a custom-made delivery system. The intra-oral device was made to fit each participant's dentition. Further, the level of gag reflex was taken into consideration for each participant in the design of the intra-oral device. A suction tube was placed across the posterior tongue near the throat to remove solution and saliva. The solution delivery system was controlled by an original computer program.

Results: Older adults presented significantly different maximum intensity timing and slope for both concentrations compared with young adults (slope for 1.5 M, $p < 0.01$; others, $p < 0.05$). No significant differences were found between the older and young adults for reaction timing and maximum intensity.

Conclusion: We conclude that older adults perceived sweetness more slowly than young adults, and ultimately perceived almost the same intensity as young adults. This is the first reported characterization of the time–intensity profile of sweet taste intensity of glucose in older adults. Using a standardized system enabled us to assess and compare feedback on taste intensities among different age groups in real-time. Based on this, we recommend older adults "savor" to perceive sweet tastes at the same level experienced by young adults.

KEYWORDS

older adult, sweet taste, glucose, time–intensity, humans

Introduction

Glucose is the primary fuel for life and cellular uptake of glucose is a fundamental process for metabolism, growth, and homeostasis (1). The adult human brain generally represents about 2% of total body mass but consumes approximately 25% of the glucose supply (1). The cognitive requirement for glucose may not change markedly with aging and older adults should consume an adequate amount of glucose daily. However, the intake of too much sugar increases the risk of obesity, diabetes, and dental caries (2–4). The World Health Organization (WHO) recommends reducing the intake of free sugars to less than 10% of total energy intake (5). To understand age-related changes in static taste perception in humans, the detection threshold (the minimum concentration at which a participant can reliably distinguish between water and taste) (6–9), the recognition threshold (the minimum concentration at which a participant can distinguish taste quality, such as sweet or salty) (10–18) and the suprathreshold (the stimulus that is large enough to produce a detectable physiological effect) have been studied (19–28). Studies using the whole-mouth method (11–16) and the filter paper disc method (17) have reported that sweet taste detection thresholds and recognition thresholds increase with age. However, three studies of sweet taste at the suprathreshold level report that the taste intensity of sweet solutions is lower in older adults than in young adults (19–21), while six studies found no significant difference (22–27).

Previous studies have attempted to characterize the temporal aspects of taste perception in young adults, in terms of time–intensity evaluations (29–36). However, there have been no previous studies of time–intensity sensory evaluation of taste in older adults except for a study of salty taste, which showed that the perception of dynamic taste intensity in older adults increases slowly (37). Hence, we hypothesized that older adults perceive sweet taste intensity more slowly than young adults.

Materials and methods

Participants

Ninety healthy adults were recruited for the study. The older adult group consisted of 40 individuals (20 men, 20 women), mean and standard deviation (SD) were 70.1 ± 6.7 in 60–85 years and body mass index (BMI), 23.0 ± 2.5 kg/m^2. The young adult group comprised 50 individuals [25 men, 25 women; age, 21–34 years (26.7 ± 3.3); BMI, 21.7 ± 3.0 kg/m^2] (**Table 1**). This study included 16 older adults and 8 young adults included in a previous study (37). This study was a preliminary study, therefore, we set the number of participants per group tentatively. For reference, the number of participants in previous sweet taste studies was 14–80 participants in cup tasting tests and filter paper disk tests (6–27), and 7–20 participants in time–intensity sensory evaluations (29–36). Advertisements around Tokyo Dental College were used to recruit participants between April 2019 and March 2022. Individuals who met at least one of the following criteria were excluded from the study: (1) smokers, (2) reported to have difficulties using the intensity meter during time–intensity recording, (3) older adults with Mini-Mental State Examination scores of 22 or less (38) and suspected dementia, and (4) people with taste, smell, psychiatric, or neurological disorders. Ten older adults had hypertension, reflux esophagitis, hyperlipidemia, and rheumatoid arthritis and took medication. The information sheets for these medications listed taste disorder as a side effect at the following incidence rates: at less than 0.1% for one medication, 0.1%–5% for one medication, less than 0.3% for one medication, 0.5%–1% for one medication, less than 1% for one medication, and unknown for six medications. However, participants on medication were not largely affected by their medication and they could distinguish the two glucose solutions of different concentrations used in this study. No enrolled participants were excluded from our study.

TABLE 1 Demographic data.

	Older	Young
Number of participants	40	50
Age, mean (SD)	70.1 (6.7)	26.7 (3.3)
Sex (male/female)	20/20	25/25
BMI, mean (SD)	23.0 (2.5)	21.7 (3.0)

SD, standard deviation; BMI, body mass index.

The participants were ordinary citizens and not specially trained. The study was conducted according to the Ethical Guidelines for Medical and Health Research Involving Human Subjects of the Ministry of Health, Labour and Welfare and the Ministry of Education, Culture, Sports, Science, and Technology, Japan and the Declaration of Helsinki on Biomedical Studies Involving Human Subjects (39). The study was approved by the Institutional Review Board of Tokyo Dental College (No. 676), and all participants provided written informed consent.

We assigned registration numbers to the data. The data were collected between April 2019 and March 2022. Correspondence tables between registration numbers and data were strictly managed. The data are not identifiable to anyone other than the registered researchers.

Taste solutions

The 0.6 M (108 g/L) and 1.5 M (270 g/L) glucose solutions were prepared with distilled water. Based on the results of a pilot study, we chose 0.6 M and 1.5 M glucose because participants could differentiate between the two concentrations (0.5 M, 0.7 M, and 1.0 M concentrations were not chosen), and they could be completely rinsed from the tongue. The 0.6 M and 1.5 M concentrations were based on estimations for soft drink and honey, respectively. We did not disclose the composition or intensity of the taste solutions to the participants.

Distilled water was used to wash out the glucose solutions and also as the control. All solutions were kept at 25°C.

Experimental design

This study comprised two experiments.

Experiment 1: cup tasting (static taste perception in the mouth).

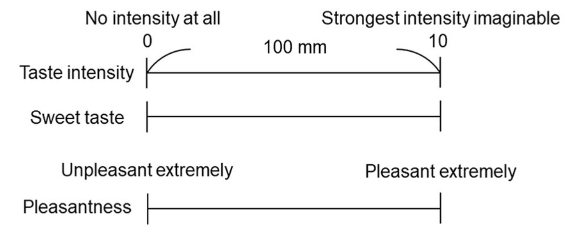

FIGURE 1
Visual analog scale (VAS) for the questionnaire. A 100 mm horizontal straight line was used to represent the VAS. Participants rated the taste intensity of the overall taste, sweet taste intensity, and pleasantness.

Experiment 2: time–intensity sensory evaluation (dynamic taste perception in the mouth).

Experiments 1 and 2 were conducted on the same day for a maximum of three participants because of the many processes involved in the experiment and the time restriction of the hospital.

Experiment 1: cup tasting

Each participant performed the cup tasting test once for each of the solutions (0.6 M and 1.5 M). Six milliliters of taste solution was sipped from an unlabeled paper cup, held in the mouth without gargling or swallowing, and spat out after 6 s. The mouth was then washed with distilled water, and the taste rating recorded on a paper sheet. Participants rated the intensity of the overall taste (sum of all taste qualities perceived), sweet taste intensity, and the pleasantness of the solution on a 0–10 visual analog scale (VAS); "0" represented "no intensity at all" and "10" represented the "strongest intensity imaginable" (Figure 1). This method with no gargling or swallowing was consistent with experiment 2, in which the solution flowed only over the tongue. Participants were provided distilled water in another paper cup to completely rinse the residual taste before receiving another solution.

Experiment 2: time–intensity sensory evaluation

For time–intensity sensory evaluation, the solution was presented to each participant's tongue using the taste solution delivery system under standardized conditions (40) (Figure 2). The intra-oral device was made to fit each participant's dentition. Ten days before the experiment, dentists took an impression of each participant's dental arch. The impression was disinfected with 0.1% sodium hypochlorite with standard precautions to prevent infection, and a working model was made. This process took 90 min. We then designed and fabricated an intra-oral device for

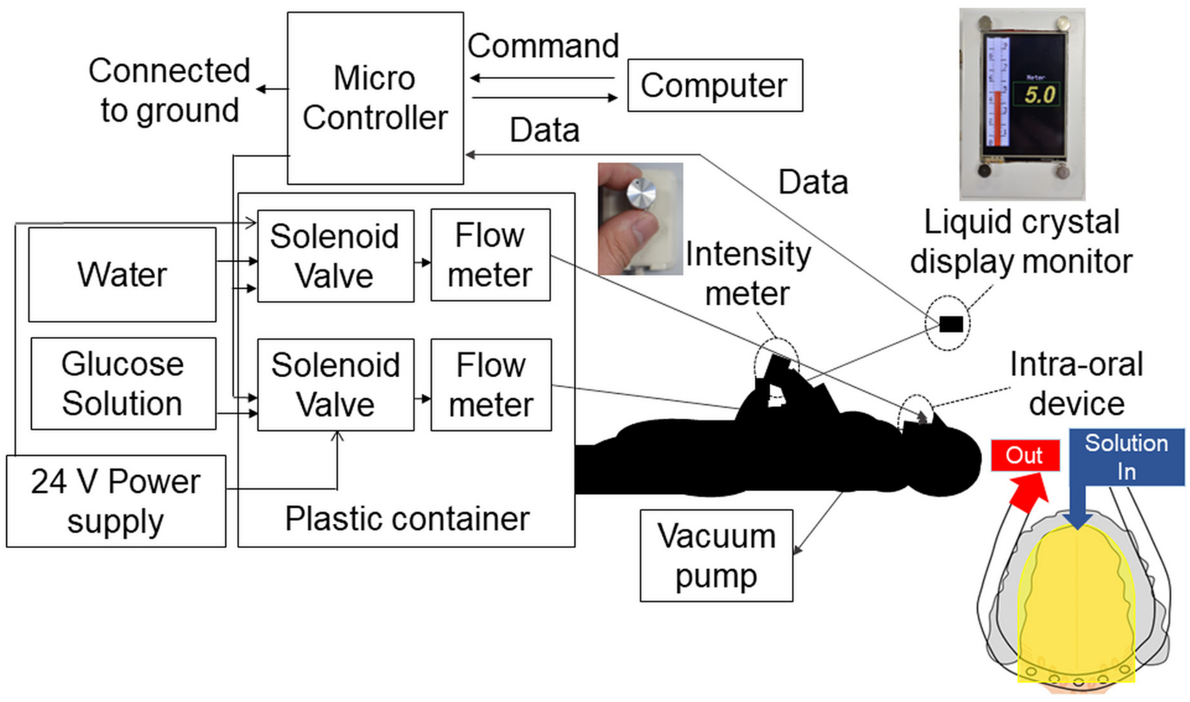

FIGURE 2
Time–intensity sensory evaluation meter and solution delivery system. The combined time–intensity sensory evaluation meter and computer-controlled taste stimulus delivery system were synchronized. The intra-oral device was made to fit each participant's dentition. Furthermore, for each participant, the level of gag reflex was taken into consideration in the design of the intra-oral device. The solution flowed to the dorsal and lateral sides of the tongue covering the fungiform and anterior half of the foliate papillae (yellow areas in the figure). The solution and saliva were removed by a suction tube placed at the back of the mouth. The overall perceived taste intensity was recorded by participants using the rotary dial on a hand-held time–intensity sensory evaluation meter during administration of the taste solution. The scale displayed on a liquid crystal display monitor corresponded to the taste intensity, ranging from 0 (no taste) to 10 (strongest taste imaginable).

each participant, with consideration of the level of gag reflex (40), which took 10 days.

The solution was supplied to the lateral and dorsal sides of the tongue and adjusted to ensure coverage of the fungiform papillae and the anterior half of the foliate papillae. A suction tube was placed across the posterior tongue near the throat to remove solution and saliva so that the participants did not need to swallow them.

The solution delivery system was controlled by an original computer program. The flow rate of solutions was constant at 110 ml/min and the participant did not feel any tactile sensations (40).

A prior study by Gotow et al. (41) found that when measuring time–intensity curves of bitterness, the perceived intensity was lower in the initial trial when compared with the second, third, and fourth trials. Additionally, the perceived intensity-time course did not differ among the second, third and fourth trials (41). Gotow et al. (41) suggested that untrained participants need a training trial using a warm-up sample before starting the test to obtain reliable performance in time–intensity evaluation (41). Therefore, at the beginning of experiment 2 we delivered distilled water onto the tongue of each participant for approximately 5 min to check the system. Warm-up samples (taste solution and water) were then each delivered once to each participant's tongue. Formal testing then commenced as described below.

A block design was employed. The experiment started with the 0.6 M glucose solution (the lower concentration solution) and we then delivered the 1.5 M solution by the same method. Only one concentration within one session was used (37). A session consists of 10 pairs of glucose and distilled water delivery for 10 s each (**Figure 3**). The 10 s stimulus duration was chosen based on studies showing that participants perceive maximum intensity of sucrose solutions between 5.0 and 10 s (29), and 6.0 s (36). The 10 s stimulation was therefore long enough to examine maximum intensity timing for 0.6 M and 1.5 M solutions. Complex methods were avoided so that older adults could concentrate on assessing taste intensity without being fatigued. We also recorded a decline in perceived sweet taste intensity during washing out. These data are not reported in this paper because the disappearance of the sweet sensation was not physiological but artificial (**Figure 3**) (37).

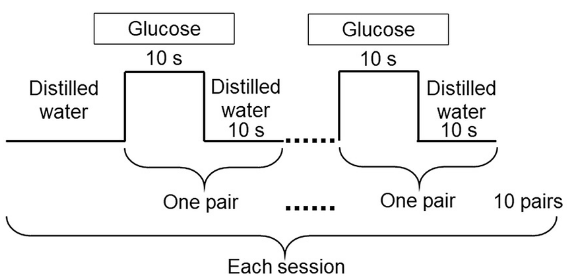

FIGURE 3
Experimental design. The experiment was carried out using 0.6 M and 1.5 M glucose solutions. Each session started with distilled water, followed by 10 pairs of glucose for 10 s, and distilled water for 10 s. Distilled water was used to wash out the glucose solutions. Only one concentration was used within each session.

The overall perceived taste intensity was recorded by participants using the rotary dial developed by Goto et al. (40) (**Figure 2**). The intraoral device and solution delivery system was also designed by Goto et al. (40). The rotary dial consisted of a variable resistor and a 13 mm diameter knob. The scale on the dial corresponded to a taste intensity, ranging from 0 (no taste) to 10 (strongest taste imaginable) (37, 40). A computer-controlled taste solution delivery system connected to the intra-oral device was synchronized with the time–intensity evaluation meter, and participants' perceptions were monitored in real-time (37, 40). In this study, we modified the system and the values recorded by the dial were displayed on a digital monitor. Older adults were less fatigued by this new design. An original computer program was written for this study.

The experimental conditions were fixed. An author (H.W.) communicated with the participants to obtain informed consent and to provide instructions for the experiments with the aid of an explanatory leaflet. Participants attended the experiments in the morning and in the same room. The room temperature was 24.0 ± 1.0°C.

Questionnaire evaluation after time–intensity sensory evaluation

Immediately after the time–intensity sensory evaluations, participants rated pleasantness, sweet taste intensity, and taste intensity (**Figure 1**).

Data analysis and statistics

We used R software 3.4.1[1] for statistical analyses. We set statistical significance at $p < 0.05$.

Taste solutions

A Wilcoxon signed-rank test was performed on the nonparametric and paired data to measure the reported differences in sweet taste between 0.6 M and 1.5 M glucose solutions. We used this test in the cup tasting test and in the time–intensity sensory evaluation.

Questionnaires

We used the Shapiro–Wilk test to investigate the normality of the data distribution. As a result, a nonparametric test was applied; the Mann–Whitney U test was used to test for significant differences between older adults and young adults in taste intensity, sweet intensity, and pleasantness. Multiple comparisons were not performed because two groups were compared according to the hypotheses.

1 www.r-project.org

Time–intensity sensory evaluation

The time–intensity profiles were analyzed with MATLAB R 2019a (The MathWorks, Inc., Natick, MA, USA). We carefully observed all profiles and included nearly all of the data. Strange profiles caused by the following operational errors were excluded from the study: (i) never turned the rotary dial during taste solution delivery, (ii) did not turn the rotary dial during taste solution delivery but did turn the rotary dial during water delivery, (iii) turned the dial more when water came onto the tongue. As a result, 1.4% (0.6 M young adults), 1.4% (1.5 M young adults), 4.3% (0.6 M older adults), and 3.0% (1.5 M older adults) of the profiles were excluded. Then, the average of all profiles and the standard error of the mean (SEM) were calculated for each condition.

The features of time–intensity profiles used for further analysis were as follows (**Figure 4**). (1) Maximum intensity (the mode of the smoothed intensity larger than 80% of the maximum smoothed intensity above the baseline; i.e., the highest and longest plateau on the profile), (2) reaction timing (the time at which the taste intensity value started to become larger than the baseline intensity), (3) maximum intensity timing (the time in milliseconds for the intensity curve to plateau), and (4) slope (the best-fit straight line was determined using linear regression from 10 selected time–intensity points) (40).

We used the Shapiro–Wilk test to test the normality of the distribution of the data. Nonparametric tests were then applied. We observed all data, and excluded strange values, which were the outliers identified through the statistical concept suggested by Tukey (42). Outliers for each participant were identified as those outside the following intervals: [Q1 − 1.5 IQR, Q3 + 1.5 IQR], where "Q" stands for "quartile" and "IQR" stands for "interquartile range" (42). We performed the Mann–Whitney U test to examine differences between older and young adult groups. Multiple comparisons were not performed.

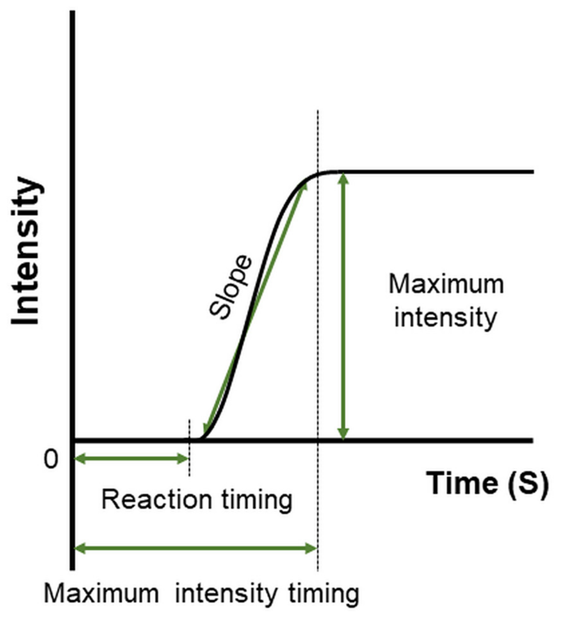

FIGURE 4
Features defined on the time–intensity profile. Reaction timing is the time at which the value of taste intensity starts to become larger than baseline intensity. The slope is the change in taste intensity per second. Maximum intensity is the highest and longest plateau on the profile. Maximum intensity timing is the time in milliseconds for the intensity curve to plateau.

Results

Experiment 1: cup tasting

Paired-test results showed that participants perceived the taste intensity of 1.5 M glucose as significantly different from that of 0.6 M glucose (older adults $p < 0.001$, young adults $p < 0.001$). Participants perceived the sweet taste stimuli and significantly differentiated between the two concentrations (**Figure 5**).

The overall taste intensity, sweet intensity, and pleasantness in the time–intensity sensory evaluation are presented in **Figure 6**. No statistically significant differences between older adults and young adults were detected for taste intensity, sweet intensity, or pleasantness (**Figure 6**).

Experiment 2: time–intensity sensory evaluation

Time–intensity profiles are shown in **Figure 7**. The results indicate that older adults perceived sweet taste more slowly than young adults.

Paired-test results showed that participants perceived the taste intensity of 1.5 M glucose as significantly different from that of 0.6 M glucose (older adults $p < 0.001$, young adults $p < 0.001$). Participants perceived the sweet taste stimuli and significantly differentiated between the two concentrations (**Figure 8**).

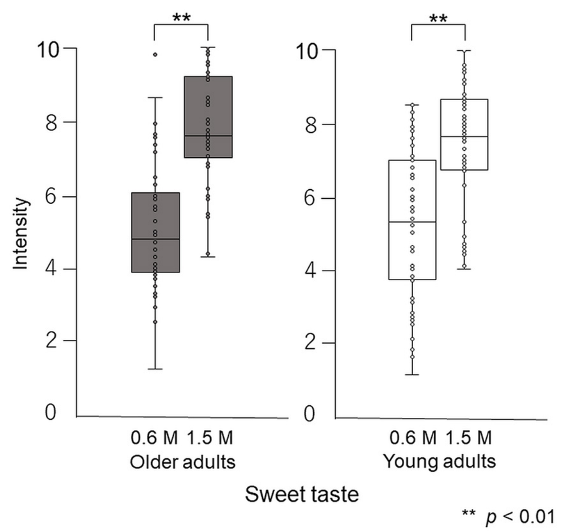

FIGURE 5
Differences in intensity between 0.6 M and 1.5 M glucose solutions in the cup tasting test (experiment 1). Both older and young adult participants perceived the taste intensity of 1.5 M glucose as significantly different from that of 0.6 M glucose ($p < 0.01$).

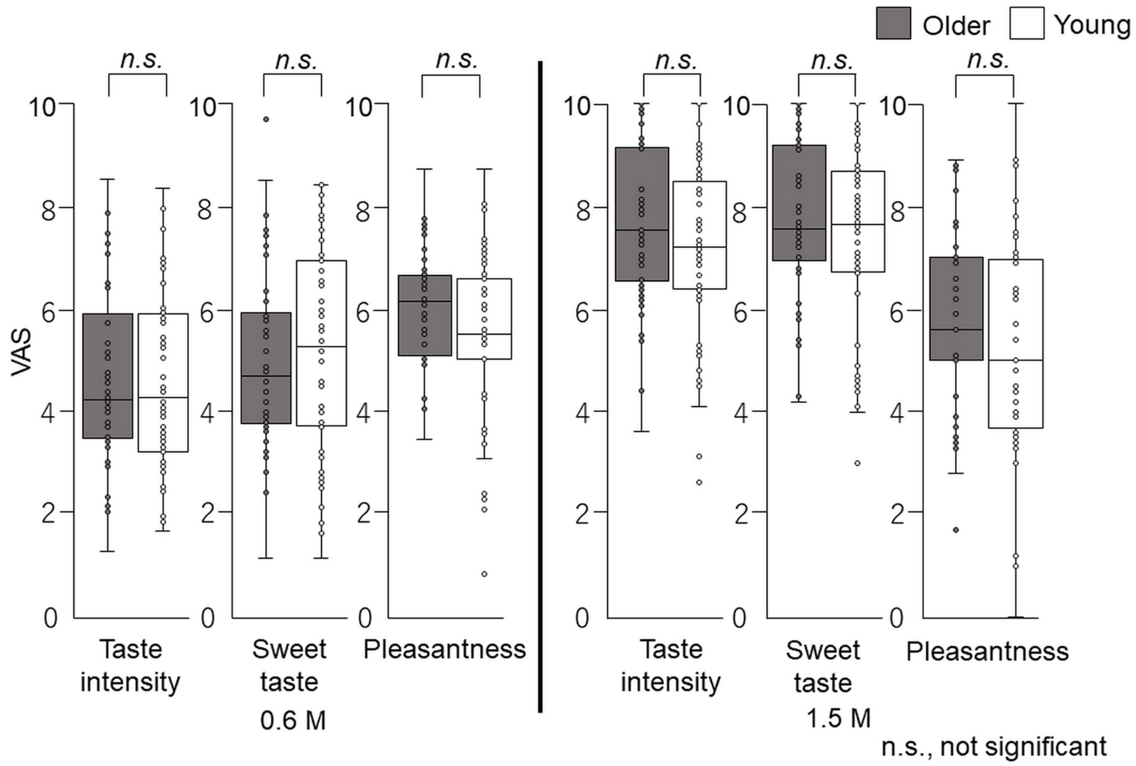

FIGURE 6
Questionnaire evaluation of the cup tasting test (experiment 1). Participants sipped the solution from a cup, held it on the tongue with no tongue movement, gargling, or swallowing, and spat it out after 6 s. The mouth was then washed with distilled water and static sweet taste intensity and pleasantness rated and recorded on a paper sheet. No significant differences were observed between older and young adults. Older adults, n = 40; young adults, n = 50.

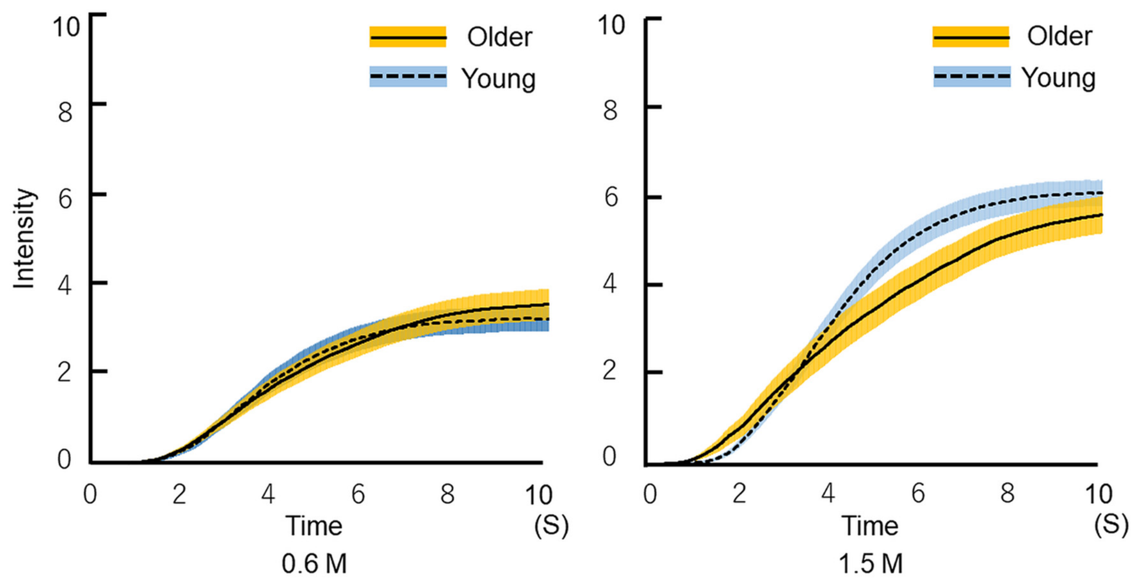

FIGURE 7
Time-intensity sensory evaluation of sweet taste intensity for all participants. The solutions were delivered to the tongue through a custom-made delivery system while participants recorded dynamic taste intensities on a hand-held time–intensity sensory evaluation meter. First, the 10 pairs of 0.6 M glucose for 10 s and distilled water for 10 s were delivered in a blocked design. Next, a 1.5 M solution was administered using the same method. We checked all time–intensity profiles. The profiles that included intensity meter operational errors were excluded. Then, the average of the replicated measurements of all profiles was calculated for each condition (the figure shows the mean ± SEM). The time required for older adults to begin to perceive sweetness was not different from that of young adults. Older adults perceived sweetness more slowly than young adults, and ultimately perceived almost the same intensity of sweetness as young adults. Older adults, n = 40; young adults, n = 50.

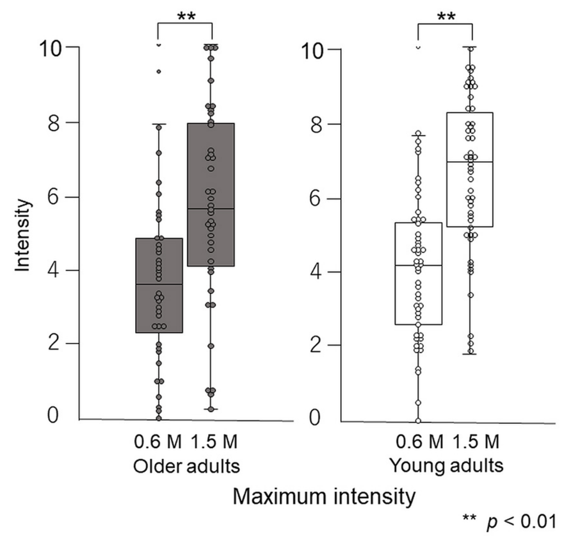

FIGURE 8
Differences in maximum intensity between 0.6 M and 1.5 M glucose solutions in time–intensity sensory evaluation. Both older and young adult participants perceived the taste intensity of 1.5 M glucose as significantly different from that of 0.6 M glucose ($p < 0.01$).

Maximum intensity

The maximum taste intensity of older adults was not significantly different from that of young adults (**Figure 9**).

Reaction timing

Older adults did not significantly differ in their reaction timing compared with young adults (**Figure 9**).

Maximum intensity timing

At both concentrations, there was a significant difference ($p = 0.01$) in the maximum intensity timing for older adults compared with young adults. For 0.6 M glucose, the median of maximum intensity timing was 7.1 (Q1 to Q3, 6.1–8.1) s for older adults and 6.4 (5.2–7.0) s for young adults. For 1.5 M glucose, the maximum intensity timing was 7.7 (6.1–8.4) s for older adults and 6.2 (5.5–7.6) s for young adults ($p = 0.02$) (**Figure 9**).

Slope

At both concentrations, the slope of older adults was significantly different from that of young adults. For 0.6 M glucose, the slope of older adults was 0.10 (0.06–0.12) intensity per second (s), which was significantly different from the 0.11 (0.08–0.16) intensity/s for young adults ($p = 0.04$). For 1.5 M glucose, the slope of older adults was 0.14 (0.09–0.19) intensity/s, which was significantly different from the 0.21 (0.13–0.27) intensity/s of young adults ($p = 0.003$) (**Figure 9**).

Questionnaire evaluation after sensory evaluation of time–intensity

The overall taste intensity, sweet intensity, and pleasantness in the time–intensity sensory evaluation are presented in **Figure 10**. No statistically significant differences in taste intensity, sweet intensity, or pleasantness between older and young adults were seen.

Discussion

In the cup tasting experiment, there were no statistically significant differences in VAS scores for taste intensity, sweet taste intensity, and pleasantness between young adults and older adults (**Figure 6**). Taste intensity findings have varied in previous reports. One reason for this is that the evaluation methods differ among studies (19–28). Therefore, to examine the differences between older and young adult groups, we used the intra-oral device and solution delivery system developed by Goto et al. (40) (**Figure 2**) and we obtained data under standardized conditions. Participants concentrated only on evaluating sweet taste intensity using an intensity meter and did not experience any stress. There are no published studies on time–intensity sensory evaluations of taste using a standardized delivery of taste solutions on the tongue for older adults.

Our results show that older adults were slower in perceiving changes in sweet intensity compared with young adults, while no significant differences were found for maximum intensities (**Figures 7**, **9**). This may result from the aging of taste receptors and/or the central nervous system. In the present study, reaction timing is thought to reflect the time that elapses between a stimulus and action potential generation by taste receptors, and maximum intensity is the frequency of action potential generation in the central nervous system. The results of this study showed no significant difference in maximum intensity between older and young adults, and the significant difference in maximum intensity timing may result from the decline of nerve conduction velocity and synaptic delay.

A study examining the number of taste buds reported that infants have 240 taste buds per whole papilla, but that the number decreases with age (43). However, a study on the number of taste buds in human fungiform papillae reported no change with age, but large individual differences (44).

The taste receptors in taste buds are activated by sugars and sweeteners and regulate glucose transport. Sweet substances are recognized by TAS1R2 and TAS1R3 (45–48). The mRNA expression levels of TAS1R2 and TAS1R3 in the fungiform and circumvallate papillae are not significantly different between young and old mice (49). Furthermore, no significant effects of aging are seen for turnover rates of taste bud cells (49). Therefore, changes in taste sensitivity with aging are not caused by aging-related degradation of peripheral taste organs (49). Experiments in rats report no age differences in the electrophysiological responses of the chorda tympani nerve (50). Taste stimuli on the tongue generate signals that are transmitted to the brain via cranial nerves (51). Considering the results from previous studies, our results may also have an association with the central nervous system.

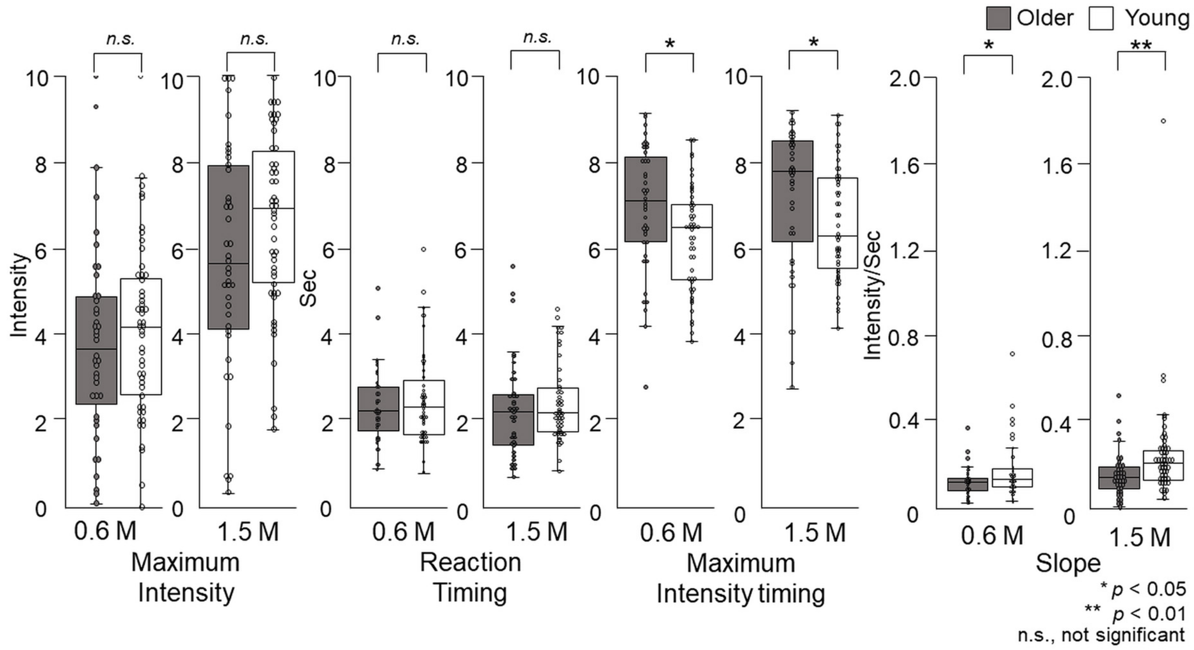

FIGURE 9
The features of the time–intensity profiles for the sweet taste of glucose. Maximum intensity, reaction timing, maximum intensity timing, and slope for the time–intensity sensory evaluation were used for further statistical analyses. Older adults did not exhibit significantly different maximum intensities or reaction timings for either concentration compared with young adults. The maximum intensity timings and slopes were significantly different between older adults and young adults for both concentrations. Older adults, $n = 40$; young adults, $n = 50$.

The time–intensity profile of sweet taste intensity of glucose in older adults has been characterized for the first time in this study. It is not possible to compare our results directly with previous studies. The results of taste intensity studies have varied in previous reports. One reason for this is that the evaluation methods differ among studies (29–36). However, using the same standardized system, we previously showed that for salty taste, older adults recognized taste intensity slowly and remained lower than that of young adults (37). However, in this study of sweet taste, only the slope and timing of maximum intensity differed significantly between the older and young adults, and there were no significant differences between older and young adults in reaction timing or maximum taste intensity (**Figure 9**). The reaction timing results show that older adults did not make slower decisions or physical responses during taste intensity evaluation. That is, older adults did not take long to perceive the sweet taste, but their recognition of taste intensity increased slowly. However, ultimately, their maximum taste intensity was not significantly different from that of young adults. Based on the results of this study, we recommend older adults "savor" to perceive sweet tastes at the same level experienced by young adults.

A limitation of this study is that the effect of medications taken by the older adults is not clear. In our literature survey, it was difficult to determine the incidence of drug-induced chemosensory disorders because functional measurements of chemosensory processes have not been performed in systematic well-controlled clinical trials (52). However, the incidence of adverse chemosensory effects from medications was 5% on average across most medications (52). In our study, 10 of the 40 older adults had taken medications for hyperlipidemia, reflux esophagitis, hypertension, and rheumatoid arthritis. The information for these medications list taste disorder as a side effect. All participants in the study distinguished between the two glucose solutions used, and the data distribution did not show any features associated with or without medication. Our data showed no significant differences in either maximum intensity or slope between older adults taking medications (10 participants) and those without medication (30 participants) ($p > 0.05$, Mann–Whitney U test). Adverse reaction from medication was not significant in our study; however, the effects of medication on taste should always be considered.

The investigators not being blind to the solution used represents a second potential limitation. We were aware of this limitation from our previous study of salty taste (37). Therefore, all authors checked all data and analyzed them repeatedly to avoid investigator bias (37). We included nearly all of the data, except for that affected by difficulties in using the intensity meter, as well as outliers identified through the statistical techniques suggested by Tukey (42).

A third potential limitation is that the participants did not receive complete sensory training. We appreciate that participant training can help reduce inter-individual variability in sensory evaluation and increase the reliability and reproducibility of the data. However, using the standardized system, participants concentrated only on the evaluation of sweet taste intensity and did not experience any stress. The participants remained motivated to participate in the series of experiments and participant bias due to fatigue was avoided.

The standardized system and thorough analysis at both personal and group levels allowed us to elicit physiological characteristics from the whole tongue. Participants perceived no

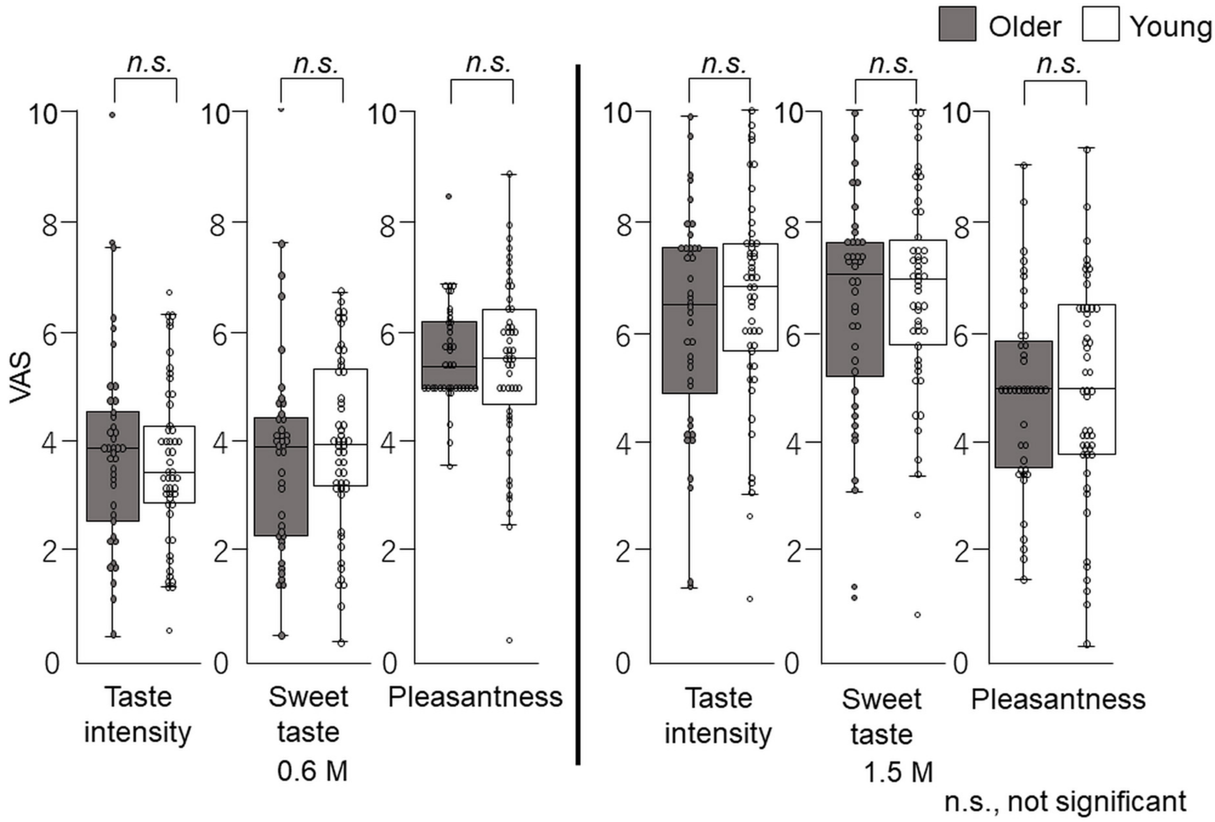

FIGURE 10
Questionnaire evaluation after time–intensity sensory evaluation (experiment 2). Immediately after the time–intensity sensory evaluation, participants reported the taste intensity, sweet taste intensity, and pleasantness perceived during the time–intensity sensory evaluation using the visual analog scale scores of the questionnaire. These results showed no significant differences in taste intensity, sweet taste intensity, or pleasantness between older and young adults for both concentrations. Older adults, *n* = 40; young adults, *n* = 50.

tactile sense and no taste from taste receptors in the pharynx or gastrointestinal tract. The approach taken in this study was safe and can be used to inform future experimental studies with older adults. The risk of aspiration was very low using this solution delivery system because the participants did not need to swallow the solution. In fact, no participants in this study experienced aspiration.

The fourth potential limitation of this study is that we did not use general labeled magnitude scales (gLMS) but used VAS scores when participants rated pleasantness, sweet taste intensity, and taste intensity, in cup tasting and immediately after the time–intensity sensory evaluations (**Figures 1**, **6**, **10**). GLMS may be better for pleasantness but we employed the same method (VAS) as that used for the intensity assessments. We chose VAS for intensity considering the specific goals and context of our project, as suggested by Hayes et al. (53). Hayes et al. (53) showed that using sucrose samples ranging from 0.19 to 0.47 M, there was no clear advantage between gLMS and VAS because participants could differentiate the intensities of the sucrose samples. In addition, gLMS data show evidence of categorical behavior while VAS data do not. Participants exhibited substantial categorical behavior, clustering their responses near the verbal labels. Moreover, providing clear written instructions to rate between adjectives was not successful in reducing this behavior (53). Participants in this study focused on taste intensity only with no semantic information. Therefore, we assumed that VAS would be appropriate in this project.

Another potential limitation of the study is that no information about concentration of the sweet solution was provided to the participants. As a result, the intensity estimates in the cup tasting test (experiment 1) might have been higher than those recorded during and immediately after the time–intensity sensory evaluation (experiment 2). Participants' comments and researchers' observations indicated that the reason for the higher intensity estimates in the cup-sipping stimulation was mainly because of the participants' first impression of sweetness. In the cup tasting test, participants tasted the 0.6 M solution for the first time. Many described it as "sweet" and gave it a high rating. During the time–intensity sensory evaluation, participants focused on perceiving the taste intensity. Other factors, such as repeated stimulations (10 times), longer stimulation time of a sweet taste, and sitting position vs. supine position, did not substantially affect participants' evaluations.

Conclusion

The time–intensity profile of sweet taste intensity of glucose in older adults has been characterized for the first time in this study.

The time–intensity profile of older adults quantitatively showed that although the reaction timing required for older adults to begin perceiving sweetness was not different from that of young adults, they perceived sweetness more slowly than young adults, and ultimately perceived almost the same intensity of sweetness as young adults. These results provide a benchmark for sweet taste perception and useful dietary advice for the general public.

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 Review Board of Tokyo Dental College (No. 676). 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

HW: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. HM: Data curation, Investigation, Methodology, Validation, Writing – review & editing. MT: Data curation, Formal analysis, Methodology, Validation, Visualization, Writing – review & editing. HS: Data curation, Funding acquisition, Investigation, Methodology, Validation, Writing – review & editing. KI: Data curation, Investigation, Validation, Writing – review & editing. AI: Data curation, Investigation, Validation, Writing – review & editing. TG: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, 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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Increased functional connectivity following ingestion of dried bonito soup

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Soup, including dried bonito broth, is customarily consumed as an umami taste during meals in Japan. Previous functional magnetic resonance imaging (fMRI) studies have investigated neuronal activation following human exposure to carbohydrates and umami substances. However, neuronal activity following ingestion of dried bonito soup has not been investigated. Additionally, recent progress in fMRI has enabled us to investigate the functional connectivity between two anatomically separated regions, such as the default mode network. In this study, we first investigated the altered functional connectivity after ingesting dried bonito soup in healthy volunteers. Functional connectivity in several brain regions, including the connection between the vermis, part of the cerebellum, and bilateral central opercular cortex, was markedly increased after ingesting dried bonito soup, compared to the ingestion of hot water. Physiological scaling showed that satiety was substantially increased by ingesting hot water rather than dried bonito soup. These results indicate that increased functional connectivity reflects the post-ingestive information pathway of dried bonito soup.

KEYWORDS

functional MRI, dried bonito soup, human, functional connectivity, vermis

1 Introduction

Soup that includes dried bonito broth provides an umami taste to Japanese foods (1). Dried bonito soup contains umami ingredients such as L-glutamate, inosine monophosphate (IMP), and amino acids, which exert anti-depressive (2) and anti-sympathetic nervous system effects (3). The ingestion of dried bonito soup ameliorates the aggressive behavior associated with perinatal dioxin exposure in children (4). In addition to behavioral and physiological studies, previous studies have investigated Fos expression in the rat brain after intragastric administration of dried bonito (5). Intragastric infusion of dried bonito increased Fos expression in the medial preoptic area, hypothalamus, and central nucleus of the amygdala, indicating that information regarding the ingested dried bonito was processed in the forebrain. Another study investigated the activity of the hypothalamus following infusion with amino acids, glucose, or lipid emulsions. The lateral hypothalamus was activated under amino acid and glucose conditions, but not under lipid emulsion condition (6).

Functional magnetic resonance imaging (fMRI) is a promising tool for noninvasive investigation of brain function. Previous studies have reported that blood oxygenation level dependent (BOLD) signal changes, which are closely linked to neuronal activation, are observed during taste (7, 8), smell (9), and food intake (10, 11). BOLD signals change in the hypothalamus after glucose intake in humans (11, 12) and rodents (13, 14). Additionally, the insular and opercular cortices are key regions for processing ingested food information (15). In contrast to glucose intake, few studies have investigated the brain response to umami substances such as L-glutamate and IMP in humans (16) and rodent models (10, 17).

Recent progress in fMRI has enabled the optimization of functional connectivity in the whole brain, which is derived from the synchronization of neuronal oscillations between anatomically-separated regions (18). Compared to local activation detected by task-based fMRI, functional connectivity includes more information on the composition of the wide brain network and complex information processing in the brain (19). Previous studies have shown that functional connectivity is related to food information processing (20) and cognitive function (21, 22). However, previous studies have focused on local activation after food intake (11) and no study has investigated the altered functional connectivity following the intake of dried bonito soup. In this study, we hypothesized that ingestion of the dried bonito soup changes the functional connectivity in the brain regions related to the processing of the information of ingested umami substance. We compared functional connectivity following the ingestion of dried bonito soup and hot water.

2 Materials and methods

2.1 Participants

Sixteen healthy volunteers (8 males and 8 females, mean age 35.9 ± 8.96 years) were recruited. All experimental procedures and protocols were approved by the Institutional Review Board of the National Institute of Advanced Industrial Science and Technology (AIST).

2.2 Visual analogue scale evaluation

The visual analogue scale (VAS) was used before each fMRI measurement according to the following three questions on hunger, satiety, and sleepiness (23). All the questions were asked in Japanese. (For example, if the hungriest state in your life is 100, how strong are your feelings of hunger now?) The participants answered the questions by placing a marker on the 140 mm line to indicate the score they felt was most appropriate for their current condition. The VAS responses were converted into hundredths of a percent and evaluated.

2.3 fMRI experiment

The experimental setup is shown in Figure 1. The VAS evaluation was performed before MRI scanning. The MRI data were acquired using a 3 T MRI system with a 32-channel brain coil (Philips Healthcare, Best, Netherlands). Structural images were acquired using a magnetization-prepared rapid gradient echo (MPRAGE): TE/TR = 5.1/11 ms, flip angle = 8°, matrix = 368 × 315 × 44, resolution = 0.70 × 0.76 × 0.70 mm^3/voxel. The fMRI was acquired to assess functional connectivity during the resting state with T2*-weighted gradient echo-planar imaging (EPI) with the following parameters: TE/TR = 30/1,500 ms, flip angle = 80°, matrix = 76 × 76 × 44, resolution = 2.5 × 2.5 × 2.5 mm^3/voxel, 420 scans (in total 10 min and 30 s). The participants were instructed to watch a cross-mark on the screen. Resting-state functional MRI and structural imaging were performed before and after ingestion.

HONDASHI® was used to create a dried bonito soup. HONDASHI® soup is one of the products of the Ajinomoto Group (Ajinomoto Co., Inc., Kawasaki, Japan) and is made with monosodium L-glutamate (32.50%), disodium 5′inosinate (2.60%), disodium succinate (0.36%), non-iodized salt, lactose, sugar, dried bonito tuna powder, bonito extract, and yeast extract.[1] The participants were asked to drink dried bonito soup, in which 3 g of dried bonito soup powder dissolved in 100 mL of hot water (approximately 40°C) or 100 mL hot water (~40°C) on a different day. Following the intake of dried bonito soup or hot water, the participants rested calmly for approximately 5 min, and the acquisition was started. The participants participated in the experiments on two separate days: one for dried bonito soup and the other for hot water. The sequence of dried bonito soup and hot water was randomly ordered for the counterbalanced design; that is, eight participants consumed dried bonito soup on the first day and eight participants consumed hot water on the first day. The gap between the first and second days was 160 ± 48 days.

2.4 fMRI analysis

2.4.1 Preprocessing of fMRI data

All MRI data were preprocessed using statistical parametric mapping software SPM12 (Welfare Trust Center for Neuroimaging, United Kingdom) to perform preprocessing steps. Preprocessing included slice timing, motion correction by realignment, and normalization of structural and functional data into a standard MNI space. Subsequently, the functional images were smoothed with an FWHM kernel of 8 × 8 × 8 mm^3.

2.4.2 Functional connectivity analysis

The head motion was checked with Artifact Detection Tools (ART).[2] The framewise displacement (FD), which is effective on the fMRI signals (24), was calculated using this tool. The outliers were detected when maximum FD >1.5 mm or 1.5° in all participants (25). Functional connectivity was then analyzed using the CONN toolbox.[3] The preprocessed fMRI data were then detrended. The mean signals in the ventricles and white matter, and six motion parameters of the head (translational and rotational motions) were regressed from the time series of each voxel to reduce the contribution of physiological noise, such as respiration and head movement. Slow periodic fluctuations were extracted using a bandpass filter (0.008–0.08 Hz). The regions of interest (ROI) were defined using the 132 anatomical regions provided by default in the CONN toolbox. The change in functional connectivity was generated for each condition (pre- and post-intake) as the product of the ROI time series multiplied by intake, and the beta weight was calculated for all ROIs. At the group level, random effects analysis was used across participants, and a *t*-test was conducted to compare ROI-based connectivity in each condition (hot water and dried bonito soup). The statistical significance of ROI–ROI

1 https://www.ajinomoto.co.th/en/our-product/food-service/hon-dashi
2 https://www.nitrc.org/projects/artifact_detect/
3 www.conn-toolbox.org

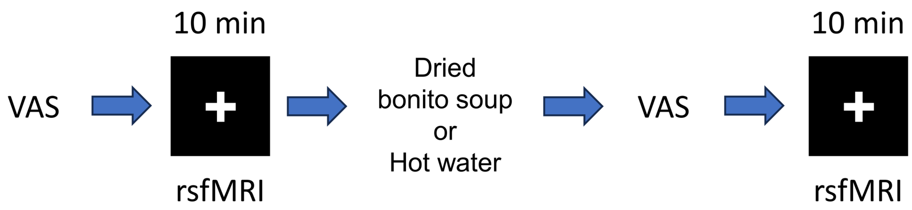

FIGURE 1
Schematic of the resting-state functional magnetic resonance imaging (fMRI). Schematic of resting-state fMRI and hot water or dried bonito soup ingestion. First, the participants answered the visual analogue scale (VAS) before first resting state fMRI. After ingestion of hot water or dried bonito soup, the participants answered the VAS again. The 2nd resting state fMRI was performed. Resting-state fMRI was performed for 10 min. The "+" mark was presented during the resting period.

connectivity was assessed using an uncorrected threshold of $p < 0.001$. Seed-based functional connectivity analysis was performed based on the results of the ROI–ROI functional connectivity, and the seeds were determined to be the vermis 6 (Ver 6), cerebellum, and bilateral central operculum (CO). The significance of seed-based functional connectivity was thresholded at $p < 0.05$, using family-wise error correction.

2.5 Statistics of VAS score

A paired *t*-test between the pre- and post-ingestion in each question and condition (dried bonito or hot water) was performed on the VAS scores following a two-way repeated measures analysis of variance. We conducted a paired *t*-test to analyze the change in the VAS ratio between pre- and post-ingestion for each condition.

3 Results

3.1 Physiological parameters

We conducted a two-factor repeated-measures analysis of variance on the conditions, and pre- and post-ingestion values were different ($F\,(1,15) = 9.525$, $p = 0.008$). We did not find any differences in the hot water and dried bonito soup conditions ($F\,(1,15) > 0.000$, $p = 0.998$) or interaction effects ($F\,(1,15) = 2.145$, $p = 0.164$).

After ingesting the dried bonito soup or hot water, hunger, satiety, and sleepiness were assessed (Figure 2). Hunger was not substantially altered by the ingestion of hot water or dried bonito soup (Figures 2A,B). The VAS scores for satiety and sleepiness increased following hot water ingestion ($p < 0.05$) (Figures 2C,E). However, ingestion of dried bonito did not markedly alter satiety or sleepiness (Figures 2D,F). Sleepiness was similar for both hot water and dried bonito soup and increased after ingestion (Figures 2E,F). A *t*-test was conducted to examine whether there was a difference in the changes in VAS before and after ingestion between the groups. The results of a paired *t*-test showed that the change was marked only in satiety ($p = 0.01$), with a greater increase in satiety for hot water than for dried bonito soup (Figure 3).

The averaged FDs were 0.119 ± 0.006 mm for dried bonito pre-ingestion, 0.117 ± 0.006 mm for dried bonito post-ingestion, 0.116 ± 0.007 mm for hot water pre-ingestion, and 0.113 ± 0.007 mm for hot water post-ingestion. There was no significant difference in FDs among the conditions ($p > 0.05$ by two-way repeated ANOVA). These results indicate that ingestion of dried bonito soup or hot water did not affect the head motion during fMRI scanning.

3.2 Increased functional connectivity following ingestion of dried bonito soup

Substantial changes in functional connectivity following the ingestion of dried bonito soup were compared with those following hot water ingestion (Figure 4A). Overall, functional connectivity with Ver 6 was increased in many brain regions, such as the motor cortex [right precentral gyrus (PreCG) and lateral sensorimotor network], temporal lobe [right Heschl's gyrus (HG), right planum temporale (PT), and bilateral central opercular cortex (CO)]. Functional connectivity between the vermis 10 and the right rostral prefrontal cortex (RPFC), which is part of the salience network, increased. Functional connectivity in the left posterior parietal cortex (PPC), which is part of the frontoparietal network, with the right hippocampus and right lingual gyrus increased. Functional connectivity increased between the left posterior part of the superior temporal gyrus (pSTG) and the right temporal occipital fusiform cortex (TOFusC). The spatial distribution of functional connectivity showed that bilateral increases were induced by Ver 6 (Figure 4B).

3.3 Seed-based functional connectivity following ingestion of dried bonito soup

We then investigated the changes in seed-based functional connectivity following the ingestion of dried bonito soup compared to the ingestion of hot water (Figure 5). Functional connectivity between Ver 6 and the bilateral CO, PreCG, TP, and middle temporal gyrus (MTG) increased substantially (Figure 5A). When the cerebellum was selected as the seed, functional connectivity with the left TOFusC and left occipital pole (OP) increased (Figure 4B). Increased functional connectivity with the bilateral CO was also observed (Figure 4C). Functional connectivity with the CO, superior frontal gyrus (SFG), cerebellum, and Ver 6 increased following the ingestion of dried bonito soup.

4 Discussion

In this study, we aimed to investigate the altered functional connectivity following the ingestion of dried bonito soup. Increased

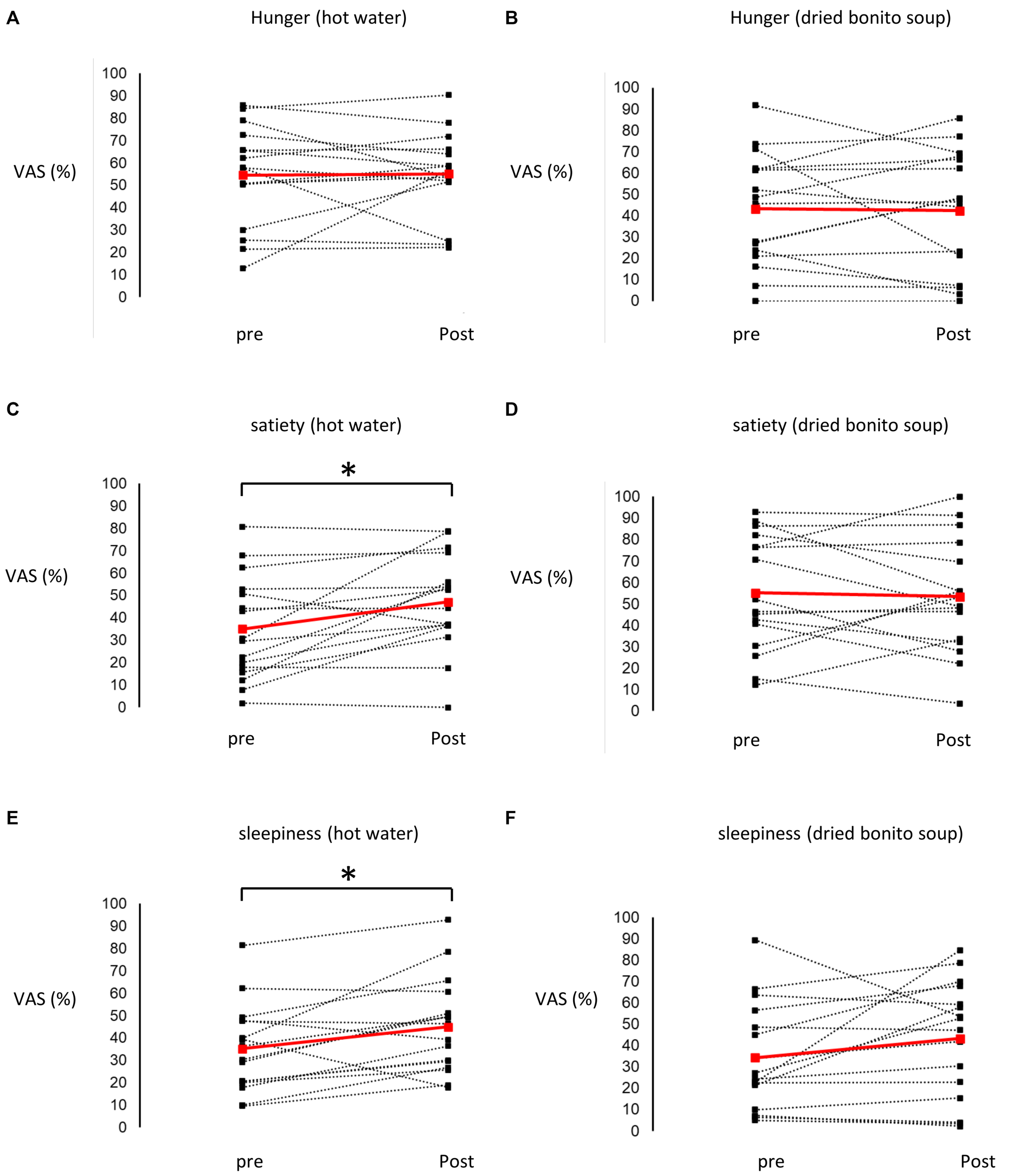

FIGURE 2
Results of the visual analogue scale (VAS) method. **(A,B)** The VAS of hunger before and after ingesting **(A)** hot water and **(B)** dried bonito soup. **(C,D)** The VAS of satiety before and after ingesting **(C)** hot water and **(D)** dried bonito soup. **(E,F)** The VAS of sleepiness before and after ingesting **(E)** hot water and **(F)** dried bonito soup. The red straight line shows the average change, and the black dashed line shows the change from pre-ingestion to post-ingestion of hot water or dried bonito soup in each participant. Statistical results are indicated using significant difference (*$p < 0.05$ between pre- and post- by paired t-test).

functional connectivity after ingesting dried bonito soup compared to hot water intake was successfully demonstrated. Functional connectivity between the vermis and several regions of the temporal lobe increased substantially. Additionally, the functional connectivity with parts of the sensorimotor and salience networks increased. Previous studies have focused on the local activation of BOLD signaling changes after ingesting nutrients such as carbohydrates and amino acids. This approach enabled the investigation of the altered neuronal activation after nutrient ingestion. However, it is impossible to investigate altered functional connectivity, which is defined as the synchronization of BOLD signal fluctuations between separate regions and is related to the processing of ingested information. This study clearly demonstrates that functional connectivity changes after the ingestion of dried bonito soup. Brain regions are key to processing ingested food information through the afferent vagus nerve.

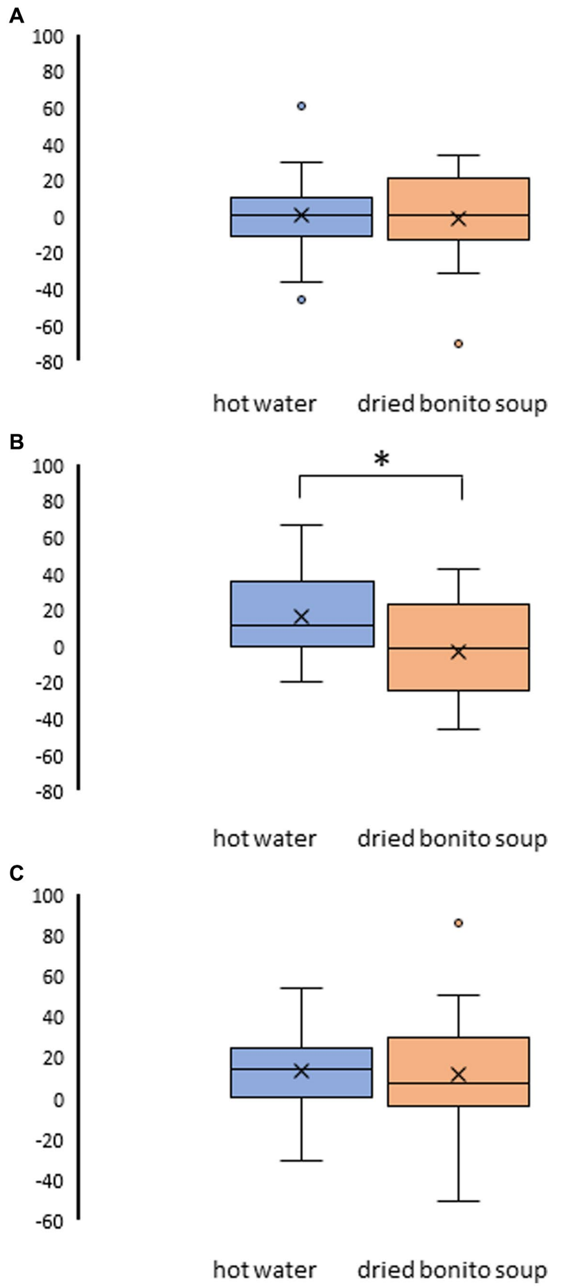

FIGURE 3
Result of the visual analogue scale (VAS) method in pre- and post-ingestion changes. **(A)** The change in VAS on hunger pre- and post-ingestion. **(B)** The change in VAS on satiety pre- and post-ingestion. **(C)** The change in VAS on sleepiness pre- and post-ingestion. The boxplots illustrate the comparison between hot water and dried bonito soup. The blue boxplot represents hot water and the orange boxplot represents dried bonito soup. The results indicate a statistical significance (*$p < 0.05$ between pre and post using paired t-test).

4.1 Role of the vermis-temporal lobe network

Functional connectivity between Ver 6 and the temporal lobes, including the bilateral CO, right HG, and right PT, was markedly increased. The operculum and vermis are involved in processing information regarding food-induced odors, such as chocolate (26). The vermis, a part of the cerebellum, is involved in the bottom-up appetitive network and plays a prominent role in feeding behavior, particularly in the drive to approach appetizing stimuli. Women with anorexia nervosa have reduced appetite and reduced responses to food images when explicitly thinking about eating food, as shown in the images. These subtypes are differentiated by increased or reduced activation in regions associated with appetitive and somatosensory impulsive responses, such as the dorsal striatum, insular cortex, and cerebellar vermis (27).

The vermis is essential for connecting the visceral organs and brain through the vagus nerve (28). Vagus nerve stimulation activates the vermis and CO (29). Rebollo et al. showed marked phase coupling between the electrogastrogram and resting-state BOLD time series in 12 nodes, called the "gastric network"; CO and Ver 6 are included in the "gastric network" (30). Ingested umami substances such as L-glutamate and IMP increase vagal nerve activity (31). These results indicate that increased functional connectivity between Ver 6 and the bilateral CO may be related to gut–brain interactions. The insular - opercular cortex is associated with spatiotemporal information regarding food intake. During meal consumption, time-locked high-frequency broadband activity at the time of food intake depends on the food types and is associated with cue-specific activity (32). The consumption of palatable foods results in greater activation of the frontal cortex and operculum/insula than the consumption of unpalatable foods (33). These results support the increased functional connectivity between the bilateral CO and the superior frontal gyrus due to seed-based functional connectivity.

4.2 Comparison between the local signal change and connectivity

Previous studies have investigated the changes in BOLD signals following the ingestion of nutrients. In contrast, the present study investigated altered functional connectivity. BOLD signal changes reflect changes in neuronal activity in local regions (34). The BOLD signal in the hypothalamus decreases substantially after ingestion of glucose (11, 35). However, compared with glucose studies, there is insufficient evidence for the neuronal imaging of umami substance ingestion. Several studies have reported that the intragastric infusion of nutrients such as umami substances and carbohydrates induces BOLD signal changes (10, 13, 14, 36). These results showed that the increased BOLD signal changes were prolonged for more than 10 min, indicating that ingested nutrients affect resting-state fMRI 5–20 min after ingestion. In contrast to BOLD signal changes, functional connectivity reflects the synchronicity of BOLD signal fluctuations between anatomically separated regions. This BOLD fluctuation is related to cognitive function (37); psychiatric diseases induce abnormal functional connectivity (38). However, no study has investigated functional connectivity after food intake.

In this study, we clearly showed altered functional connectivity related to the gut-brain axis and food intake. To our knowledge, this is the first study to demonstrate the relationship between functional connectivity and food intake in humans. A rat study revealed that functional connectivity is decreased by sucrose intake (39). The *ad libitum*-fed rats showed a trend toward higher functional connectivity than food-restricted rats. Functional connectivity is affected by the nutritional status of the body. Functional connectivity from the

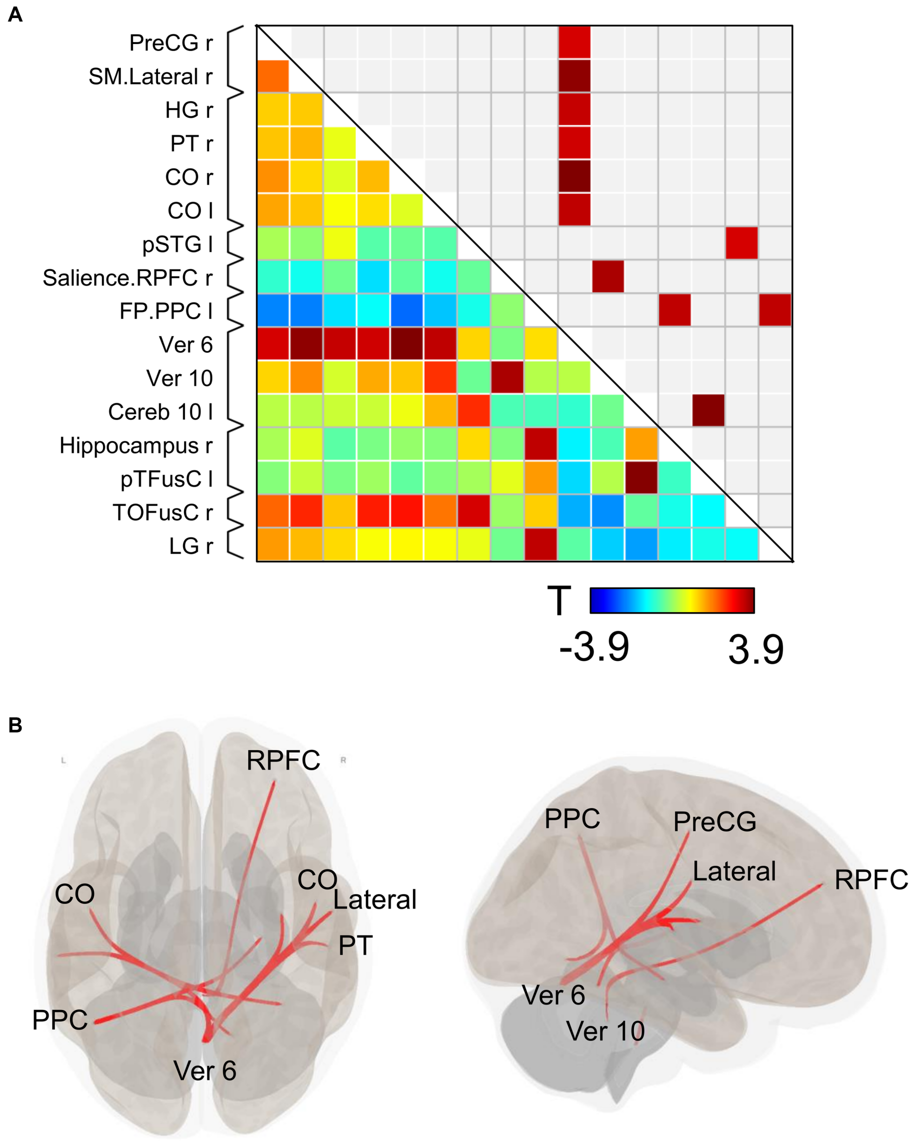

FIGURE 4
Connectivity matrix and glass brain. **(A)** Represents the functional connectivity of the regions of interest. The elements indicate the strength of connectivity between regions. In **(B)**, regions with strong connectivity are spatially represented by a glass brain. PreCG, the precentral gyrus; SM lateral, lateral sensorimotor network; HG, Heschl's gyrus; PT, planum temporale; CO, central opercular cortex; pSTG, posterior part of the superior temporal gyrus; RPFC, right rostral prefrontal cortex; PPC, posterior parietal cortex; Ver 6, vermis 6; Ver 10, vermis 10; Cereb 10, cerebellum 10; pTFusC, posterior part of temporal fusiform cortex; TOFusC, temporal occipital fusiform cortex; LG, lingual gyrus.

posterior to the anterior insula is strengthened by hunger compared to satiety, and glucose intake alters functional connectivity (40). In a mouse study, food deprivation increased the functional connectivity between the audiovisual cortex, hippocampus, and retrosplenial cortex (41). These studies indicate that functional connectivity is influenced by the nutrient state and ingested food. Therefore, the altered functional connectivity due to dried bonito soup intake is reasonable and provides insights into the physiological significance of dried bonito soup in Japanese cuisine. However, dried bonito soup contains several food ingredients, such as salt, and more precise studies using L-glutamate or IMP should be performed in the future. The objective of this study was to investigate the altered functional connectivity following the ingestion of dried bonito soup. This may have been caused by taste, ingestion, and post-ingestive effects. In the future, the effects of taste, smell, and post-ingestion of dried bonito soup should be investigated separately to understand the mechanisms of altered functional connectivity.

4.3 The limitations of this study

We investigated the functional MRI (fMRI) data of 16 participants. Although previous studies showed good quality results and the results of increased BOLD areas were replicable with less than 20 participants (15, 42, 43), there was limited statistical power in this study due to the small sample size (44). Marek et al. (44) used the big data (50,000 individuals) to estimate the sample size for improving replication rates and decreasing effect size inflation. Other studies estimate the sample

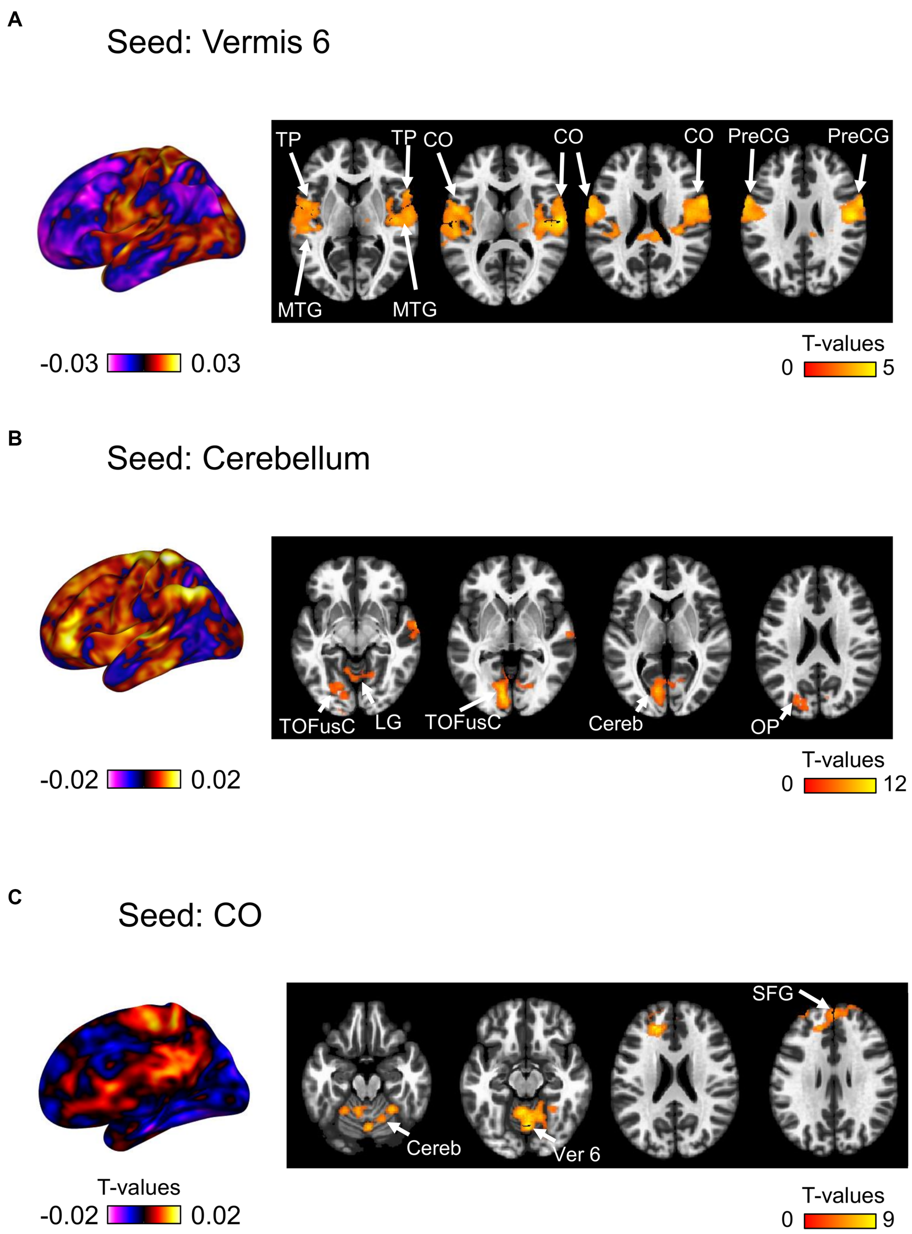

FIGURE 5
Seed-based functional connectivity. This represents the functional connectivity of each of the three regions as seeds. **(A)** Functional connectivity of vermis 6 as a seed, **(B)** cerebellum, and **(C)** central opercular cortex. The color bar indicates the *t*-value.

size for reliable reproducibility and 40–300 individuals are required (45, 46). Future studies should use larger sample sizes to assess the reproducibility of results.

In the present study, we selected the VAS method to measure the participants' feelings of hunger, and sleepiness because it has been used in the previous fMRI study (45, 47, 48). Although there is no difference in the resolving power between VAS and general labeled magnitude scale (gLMS) (49), previous studies have used the gLMS to measure the perceptual intensity, such as taste, smell, and hunger (50–52). We will compare the VAS and gLMS to measure the physiological state.

In the present study, we did not calculate the brain response to the intake of dried bonito soup because the intake of dried bonito soup or hot water was performed outside of the MRI bore to avoid the motion artifacts in ingestion. Therefore, we could not directly compare the relationship between local neuronal activation and altered functional connectivity with dried bonito soup. Future studies should attempt to compare them using fMRI by conducting ingestion in the MRI bore.

5 Conclusion

In conclusion, our study showed that dried bonito soup ingestion increased the functional connectivity in the regions involved in the information processing of ingested food, such as the vermis, central opercular cortex, a part of sensorimotor and temporal lobes. These results indicate that functional connectivity can be a marker of ingested food information in the brain.

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 Institutional Review Board of the National Institute of Advanced Industrial Science and Technology. 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

TS: Data curation, Formal analysis, Investigation, Visualization, Writing – original draft. AT: Data curation, Investigation, Writing – original draft. KO: Data curation, Investigation, Writing – review & editing. KK: Writing – review & editing. TT: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, 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.

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Macronutrient intake is associated with intelligence and neural development in adolescents

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Introduction: Macronutrient intake can be one of the most influential factors in cognitive and neural development in adolescents. Adolescence is a specific period of cognitive and neural development, and nutritional effects during this period could be life-long. Therefore, understanding the effects of macronutrient intake on cognitive and neural development in adolescents is crucially important. We thus examined the association across macronutrient intake, intelligence, and neural development using population-based cohort data.

Methods: We conducted two studies. In study 1, we included a total of 1,734 participants (boys, 907, age [mean ± standard deviation] 171.9 ± 3.44 months; range 163.0–186.0 months) from the Tokyo TEEN Cohort (TTC) to examine the association between macronutrient intake and intelligence quotient (IQ). In study 2, we included a total of 63 participants (boys, 38, age 174.4 ± 7.7 months; range 160.7–191.6 months) to investigate the effect of nutrition intake on neural development using graph theory analysis for resting-state functional magnetic resonance imaging (rs-fMRI) derived from a subset of the TTC.

Results: TTC data revealed that a higher IQ was associated in boys with increased protein intake ($\beta = 0.068$, $p = 0.031$), and in girls, with reduced carbohydrate intake ($\beta = -0.076$, $p = 0.024$). Graph theory analysis for rs-fMRI at approximately age 12 has shown that impaired local efficiency in the left inferior frontal gyrus was associated with higher carbohydrate and fat intake ([x, y, z] = [−51, 23, 8], $p_{FDR\text{-}corrected} = 0.00018$ and 0.02290, respectively), whereas increased betweenness centrality in the left middle temporal gyrus was associated with higher carbohydrate, fat, and protein intake ([x, y, z] = [−61, −43, −13], $p_{FDR\text{-}corrected} = 0.0027$, 0.0029, and 0.00075, respectively). Moreover, we identified a significant moderating effect of fat and protein intake on the relationship between change in *betweenness centrality* over a 2-year measurement gap in the left middle temporal gyrus and intelligence ($\beta = 12.41$, $p = 0.0457$; $\beta = 12.12$, $p = 0.0401$, respectively).

Conclusion: Our study showed the association between macronutrient intake and neural development related to intelligence in early adolescents. Appropriate nutritional intake would be a key factor for healthy cognitive and neural development.

KEYWORDS

macronutrient intake, adolescents, neural development, intelligence, resting-state functional magnetic resonance imaging

1 Introduction

During adolescence, the neural network undergoes a fundamental reorganization (1) related to development and maturation of cognitive functions (1, 2). Recent neuroimaging studies have illuminated structural and functional connectivity changes occurring during the transition from childhood to young adulthood, beginning in the primary sensorimotor areas and moving to the higher-order association areas, such as the prefrontal cortex and lateral temporal cortex, that mirror improvement in cognitive abilities (3). In addition, the manifestation of age-related neural reorganization could differ between boys and girls (4), leading to sexual differences in neural and cognitive development (5–7).

Nutrition intake and diet may be one of the most influential factors in neural development (8), cognitive functions, and academic achievement in children (8, 9), among various physical and social environmental factors, such as urbanicity, socio-economic characteristics, and the experience of racism (10). For instance, the effects of several specific nutrients, including omega-3 polyunsaturated fatty acids, iron, and vitamin B12 on brain development and cognitive functions have been shown (11). Research clarifying the effects of macronutrients (such as carbohydrates, fat, and protein) and dietary patterns on development of neural and cognitive functions in children and adolescents has shown that increased fat consumption was significantly associated with decreased hippocampal volume in healthy children (12); the intake of healthier foods (such as whole grains, fish, fruits and/or vegetables) was positively associated with enhanced executive function, whereas that of less-healthy snack foods, sugar-sweetened beverages, and red/processed meats was negatively associated with executive function among children and adolescents (13). In concert with various environmental factors—such as parental aggressiveness or hyperactivity and low education or income status—high fat/sugar intake may contribute to poor inhibitory control (14). A large population-based prospective cohort study showed that high intake of snack and processed foods at age 1 and age 8 years was negatively correlated with global brain volumes at age 10 years, while global brain volumes mediated the relationships between dietary patterns at ages 1 and 8 years and children's IQ at age 13 years (15). This study indicated that unhealthy dietary patterns such as the western diet—rich in saturated fats and refined carbohydrates—can impair brain development and cognitive functions in children. In addition, a diet high in protein led to improved cognitive ability of children (16, 17). Further, excess body mass—often caused by overconsumption of foods high in fat and sugar (18)—is associated with poor executive functions in children (19). Therefore, macronutrient intake would mediate brain development and cognitive functions in children.

Because intelligence can be defined as the mental ability to integrate various cognitive functions for reasoning, problem solving, and learning (20), different cognitive functions are associated with intelligence. Given this nature of intelligence, various neural circuits are involved in intelligence (20). For instance, a neuroimaging study in children has shown that higher intelligence quotient (IQ) is associated with greater neural connectivity in the attention system, while lower IQ was related to greater neural connectivity in the default mode, emotion, and language systems, after controlling for the effects of age, sex, and age-by-sex interaction (4). Macronutrient intake may influence the neural development underlying intelligence and, given sex differences in neural and cognitive development (4–7), the impact of nutrient intake on this neural development could differ between boys and girls. The adolescent period is a narrow window of neural and cognitive development, during which nutritional effects on neural and cognitive development could be prolonged or life-long. Therefore, while understanding the influence of macronutrient intake on neural development and intelligence in children is critical, research focusing on the relationship between macronutrient intake and neural development or intelligence in children is scarce. Here, we aimed to examine the associations across macronutrient intake, intelligence, and neural development in children aged 12–15 years using an adolescent cohort and resting-state functional magnetic resonance imaging (rs-fMRI) data from subsamples of the cohort.

We hypothesized that—since increased sugar and fat intake were associated with declined cognitive functions (12–14)—higher carbohydrate and fat intake would negatively impact intelligence or neural development associated with intelligence; conversely, given that protein intake was positively related to intelligence (16, 17), higher protein intake would positively impact intelligence or greater neural development. In addition, such impact of macronutrient intake on intelligence or neural development would differ between boys and girls, given sexually determined differences in neural and cognitive development (4, 5, 7).

Among various adaptations to computational techniques for neuroimaging data to capture brain network development (21), we adapted graph theoretical analysis in this study to examine functional connectivity patterns (22). Graph theoretical analysis has recently played a significant role in understanding complex brain connectivity architecture, and can provide access to topological properties that characterize the local and global architecture of the entire brain network connectivity (23). Graph theoretical analysis is thus favorable to examine associations between macronutrient intake and whole-brain neural networks.

2 Materials and methods

2.1 Study design

We conducted two studies. In study 1, we examined the association between macronutrient intake and intelligence using data from the Tokyo TEEN Cohort (TTC) study, a current prospective population-based cohort study that aims to investigate the developmental trajectory of adolescents (24). We performed study 2 to investigate the effect of nutrition intake on neural development using rs-fMRI data derived from a subset brain imaging cohort [the population-neuroscience study of the TTC (pn-TTC)] (25).

2.2 Study 1: associations between macronutrient intake and intelligence

2.2.1 Participants

We randomly extracted children born between September 2002 and August 2004 in three local governments in Tokyo (Setagaya, Mitaka, and Chofu) using the Basic Resident Register when the children were 10 years old. Trained interviewers collected data used in this study during home visits when the children were aged 12 (2nd wave) and 14 (3rd wave), achieving a follow-up rate of 94.8% at age 12 ($n = 3{,}007$) and 84.1% at age 14 ($n = 2{,}667$). We excluded participants for whom data on weight, height, dietary history, intelligence, household income, age, and sex were missing. Thus, in this study, we included a total of 1,734 participants (boys, 907: girls 827) (Table 1; Figure 1). All procedures were approved by the ethics committees of three research institutes: Tokyo Metropolitan Institute of Medical Science (approval number: 12–35); The University of Tokyo (10057); and SOKENDAI (The Graduate University for Advanced Studies; 2,012,002). We obtained written informed consent from the children's primary caregivers and informed assent from the children during each wave of the study.

2.2.2 Measures

We estimated the children's general intellectual ability at age 12 by a Full Scale IQ (FIQ) of the Wechsler Intelligence Scale for Children (WISC-III) (26).

We estimated macronutrient and calorie intake at age 14 using a brief self-administered dietary history questionnaire (BDHQ) for Japanese children and adolescents (BDHQ-15y) (27, 28). The BDHQ-15y assesses the quantities of nutrients habitually consumed from foods usually eaten (excluding intake from dietary supplements) as individual units, and is designed to obtain each individual's nutrient intake, food intake, and information based on a few markers of dietary behaviors. According to a previous longitudinal cohort study, leaving the parental home and completing education were identified as significant factors contributing to changes in diet, including an increase in the consumption of confectionery and sugar-sweetened beverages (29). Thus, it could be assumed that the dietary habits of 12-year-old children (measured for intelligence) and 14-year-old children (measured for dietary habits), who typically live at home and attend compulsory education in Japan, would not differ significantly.

Because socioeconomic status (SES) can influence executive function and brain development in children (30), we assessed annual household income by the self-report questionnaire. Annual household income was divided into 11 categories. 1: 0–990,000 Japanese Yen [JPY]/year, 2: 1,000,000–1,990,000 JPY/year, 3: 2,000,000–2,990,000 JPY/year, 4: 3,000,000 –3,990,000 JPY/year, 5: 4,000,000–4,990,000 JPY/year, 6: 5,000,000–5,990,000 JPY/year, 7: 6,000,000–6,990,000 JPY/year, 8: 7,000,000–7,990,000 JPY/year, 9: 8,000,000–8,990,000 JPY/year, 10: 9,000,000–9,990,000 JPY/year, 11: 10,000,000 and more JPY/year.

At the 2nd and 3rd waves, trained interviewers measured each participant's height and weight. We then calculated body mass index (BMI) for age (BMI-for-age),—a child's BMI for their age and sex relative to the reference population, using the WHO criteria (31).

For this study, we did not include parental educational level as a potential confounder because it can be highly correlated with household income, and a previous large cohort study in adolescents ($n = 3{,}892$ children) has shown that among the socioeconomic factors, including parental education and family income, family income best explains individual differences in cognitive test scores, cortical volume, and thickness (32). In addition, although certain environmental factors such as school type and local environment may influence cognitive development (10), this cohort study randomly

TABLE 1 Demographics of participants in study 1.

	All participants ($n = 1734$) Mean ± S.D. (range)	Boys ($n = 907$) Mean ± S.D. (range)	Girls ($n = 827$) Mean ± S.D. (range)	Sex differences	
				p-value	Wilcoxon statistic
Age (months) at 3rd wave	171.89 ± 3.44 (163.00–186.00)	171.87 ± 3.54 (163.00–183.00)	171.91 ± 3.34 (164.00–186.00)	0.47	374,294
BM-for-age (z-value) at 3rd wave	−0.16 ± 0.94 (−3.79–2.90)	−0.21 ± 1.03 (−3.79–2.90)	−0.12 ± 0.82 (−3.16–2.41)	0.009	350,250
Full Scale IQ at 2nd wave	111.26 ± 14.82 (43.79–152.30)	110.31 ± 15.30 (43.79–149.65)	112.30 ± 14.22 (51.82–152.30)	0.003	346,283
Socioeconomic status*	8.12 ± 2.61 (1.00–11.00)	8.04 ± 2.70 (1.00–11.00)	8.21 ± 2.50 (1.00–11.00)	0.41	366,519
Macronutrient intake at 3rd wave					
Carbohydrate (g/day)	350.56 ± 155.95 (66.52–1375.75)	399.49 ± 170.05 (66.52–1375.75)	296.90 ± 117.46 (96.52–1306.08)	1.0	535,141
Fat (g/day)	81.09 ± 30.64 (12.80–278.99)	86.25 ± 32.45 (20.36–278.99)	75.44 ± 27.47 (12.80–237.62)	1.0	454,585
Protein (g/day)	85.48 ± 32.73 (18.94–266.98)	93.23 ± 34.67 (20.31–266.98)	76.98 ± 28.12 (18.94–236.42)	1.0	491,862
Total calorie intake at 3rd wave (kcal/day)	2509.58 ± 950.64 (2333.33–2766.67)	2791.14 ± 1023.12 (569.68–8591.68)	2200.78 ± 751.91 (639.42–7,637)	1.0	520,607

*Socioeconomic status is measured as annual household income divided into 11 categories. 1: 0–990,000 Japanese Yen [JPY]/year, 2: 1,000,000–1,990,000 JPY/year, 3: 2,000,000–2,990,000 JPY/year, 4: 3,000,000–3,990,000 JPY/year, 5: 4,000,000–4,990,000 JPY/year, 6: 5,000,000–5,990,000 JPY/year, 7: 6,000,000–6,990,000 JPY/year, 8: 7,000,000–7,990,000 JPY/year, 9: 8,000,000–8,990,000 JPY/year, 10: 9,000,000–9,990,000 JPY/year, 11: 10,000,000 and more JPY/year. IQ, intelligence quotient; S.D., standard deviation.

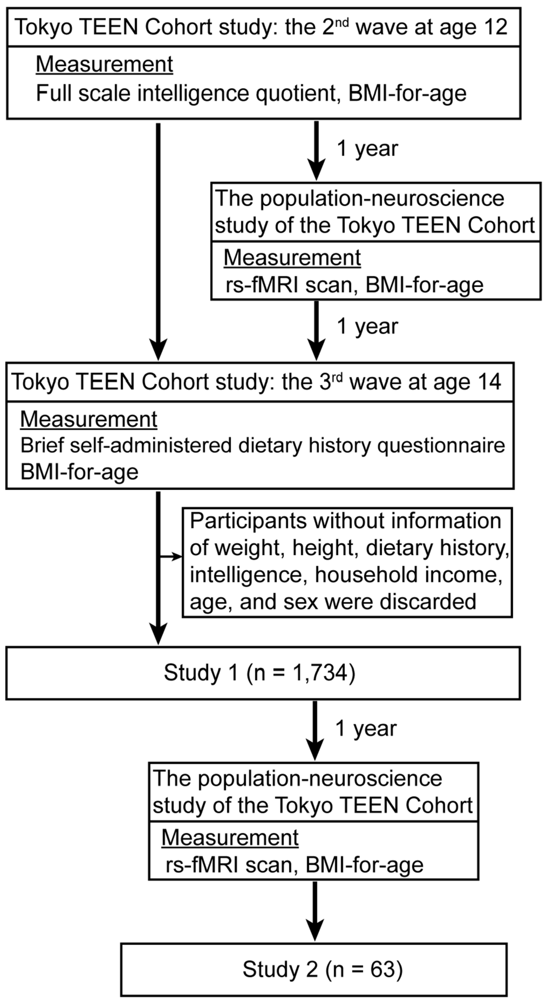

FIGURE 1
Study protocol of study 1 and 2. For study 1 (*n* = 1,734), we excluded participants for whom data on weight, height, dietary history, intelligence, household income, age, and sex were missing. For study 2 (*n* = 63), we used rs-fMRI data at the 2nd and 3rd waves, excluding participants without data for dietary history, intelligence, household income, age, and sex.

selected children from three local governments in Tokyo (Setagaya, Mitaka, and Chofu). Therefore, it is unlikely that there would be significant variation in school type or learning environment within this cohort. As a result, these potential confounding variables were not incorporated into the primary statistical analysis.

2.2.3 Statistics

We performed all statistical analyses using R statistical software (v4.3.0; R Foundation for Statistical Computing, Vienna, Austria). First, we performed Z-score normalization for macronutrient intake, total calorie intake, the measurement gap between FIQ and nutrition intake measures (months), difference between BMI-for-age at age 12 and age 14, SES, and FIQ by subtracting the mean and dividing by the standard deviation. Then, to examine associations between macronutrient intake and intelligence, we performed multiple linear regression analysis using normalized variables. We set the FIQ as a dependent variable, and carbohydrate, fat, and protein intake, as explanatory variables. In addition, we set total calorie intake, the measurement gap between FIQ and nutrition intake measures, difference between BMI-for-age at age 12 and age 14, SES, and sex as potential confounders.

Multiple linear regression analysis assumes that independent variables are not highly correlated with each other, but the variance inflation factor (VIF) for carbohydrate, fat, and protein intake and total calorie intake were 1367.22, 242.36, 69.00, and 3007.77, respectively, showing a high correlation. To address this multicollinearity problem, we created three models—model 1, dependent variable: FIQ, explanatory variable: carbohydrate intake; model 2, dependent variable: FIQ, explanatory variable: fat intake; and model 3, dependent variable: FIQ, explanatory variable: protein intake. All three models also included the measurement gap between FIQ and nutrition intake measures, difference between BMI-for-age at age 12 and at age 14, SES, and sex as potential confounders.

Because sex differences in neural and cognitive development exist during adolescence (4, 5, 7), we performed multiple linear regression analysis using the above three models in boys and girls, separately.

2.3 Study 2: associations between macronutrient intake and neural connection

2.3.1 Participants

We subsampled and enrolled some participants from the TTC (25) in study 2. We regarded participants in the TTC who showed interest in the pn-TTC study as candidate participants and enrolled some of them approximately one year later in the pn-TTC study. We used rs-fMRI data at the 2nd and 3rd waves, excluding participants without data for dietary history, intelligence, household income, age, and sex. We included a total of 63 participants (boys, 38: girls 25) in this study (Table 2; Figure 1).

2.3.2 Magnetic resonance imaging data acquisition

All participants underwent rs-fMRI sessions at the 2nd and the 3rd waves. We instructed them to remain still and gaze at a black cross on a white screen during the scanning, for one run (10 min 10 s) at the 2nd wave, and four runs (5 min 36 s each) at the 3rd wave.

We acquired all MR images using a MAGNETOM Prisma 3.0 Tesla scanner (Siemens Healthineers, Erlangen, Germany). At the 2nd wave, we used a 64-channel head coil and acquired anatomical images using a T1-weighted protocol (repetition time (TR) = 1900 ms, echo time (TE) = 2.53 ms, flip angle (FA) = 9°, field-of-view (FOV) = 256 × 256 mm^2, and resolution 1.0 × 1.0 × 1.0 mm^3). For functional images, we acquired T2*-weighted images using gradient-echo echo-planar imaging (EPI) (TR = 2,500 ms, TE = 30 ms, 38 slices, FA = 80°, FOV = 212 × 212 mm^2, and resolution = 3.3 × 3.3 × 4.0 mm^3) in ascending order. At the 3rd wave, we used a 32-channel head coil and acquired anatomical images using a T1-weighted protocol (TR = 2,400 ms, TE = 2.22 ms, FA = 8°, FOV = 256 × 256 mm^2, resolution 0.8 × 0.8 × 0.8 mm^3). For functional images, we acquired T2*-weighted images using EPI with a multiband acceleration factor 8 and generalized, auto-calibrating (TR = 800 ms, TE = 37 ms, 72 slices, FA = 52°, FOV = 208 × 208 mm^2, and resolution = 2.0 × 2.0 × 2.0 mm^3) using an interleaved manner to reduce the cross-talk of the slice selection pulse.

TABLE 2 Demographics of participants in study 2.

	All participants ($n = 63$) Mean ± S.D. (range)	Boys ($n = 38$) Mean ± S.D. (range)	Girls ($n = 25$) Mean ± S.D. (range)	Sex differences	
				p-value	Wilcoxon statistic/ *t*-value
2nd wave					
Age at rs-fMRI scan (months)	174.41 ± 7.65 (160.68–191.64)	173.63 ± 7.68 (162.36–188.04)	175.60 ± 7.60 (160.68–191.64)	0.32[#]	−1.00[#]
BM-for-age at rs-fMRI scan (z-value)[%]	−0.05 ± 0.87 (−2.12–2.49)	0.09 ± 0.97 (−2.12–2.49)	−0.25 ± 0.68 (−0.98–1.08)	0.95	479
Full Scale IQ	110.84 ± 14.24 (79.52–141.09)	110.68 ± 14.22 (84.77–141.09)	111.07 ± 14.56 (79.52–136.84)	0.92[#]	−0.11[#]
Socioeconomic status*	7.83 ± 2.73 (1.00–11.00)	7.53 ± 2.98 (1.00–11.00)	8.28 ± 2.28 (4.00–11.00)	0.37	412
3rd wave					
Age at rs-fMRI scan (months)	194.25 ± 7.52 (179.40–208.20)	193.81 ± 7.21 (179.88–207.24)	194.91 ± 8.08 (179.40–208.20)	0.58[#]	−0.56[#]
BM-for-age at rs-fMRI scan (z-value)	−0.20 ± 0.96 (−2.83–2.44)	−0.23 ± 1.09 (−2.83–2.44)	−0.14 ± 0.75 (−1.33–1.58)	0.72[#]	−0.35[#]
Macronutrient intake					
Carbohydrate (g/day)	375.22 ± 177.55 (108.79–1302.88)	433.60 ± 197.13 (172.65–1302.88)	286.49 ± 89.08 (108.79–447.99)	1.0	730
Fat (g/day)	87.64 ± 34.00 (30.07–259.89)	95.29 ± 38.49 (51.16–259.89)	76.01 ± 21.65 (30.07–132.73)	1.0	623
Protein (g/day)	92.67 ± 36.01 (34.01–241.60)	102.61 ± 39.13 (55.63–241.60)	77.56 ± 24.39 (34.01–119.46)	1.0	664
Total calorie intake (kcal/day)	2698.34 ± 1104.55 (856.91–8591.68)	3047.65 ± 1225.28 (1430.00–8591.68)	2167.39 ± 591.92 (856.91–3111.21)	1.0	727

*Socioeconomic status is measured as annual household income divided into 11 categories. 1: 0–990,000 Japanese Yen [JPY]/year, 2: 1,000,000–1,990,000 JPY/year, 3: 2,000,000–2,990,000 JPY/year, 4: 3,000,000 –3,990,000 JPY/year, 5: 4,000,000–4,990,000 JPY/year, 6: 5,000,000–5,990,000 JPY/year, 7: 6,000,000–6,990,000 JPY/year, 8: 7,000,000–7,990,000 JPY/year, 9: 8,000,000–8,990,000 JPY/year, 10: 9,000,000–9,990,000 JPY/year, 11: 10,000,000 and more JPY/year. [#]Statistical results of Student's *t*-test. [%]Seven participants do not have BMI-for-age data. IQ, intelligence quotient, S.D., standard deviation.

2.3.3 Image preprocessing

We preprocessed all rs-fMRI data using Statistical Parametric Mapping 12 (SPM12) software with the CONN functional connectivity toolbox (CONN, version 22a) (33). For rs-fMRI data at the 2nd and 3rd waves, we performed conventional preprocessing including slice-timing correction, realignment and unwarping, denoising, normalization (onto the standard Montreal Neurological Institute and Hospital space), and smoothing (6-mm full-width at half-maximum Gaussian filter) using the default parameter settings of SPM12. We estimated six motion parameters (three rotation and three translation parameters) during the realignment step. We then used the Artifact Detection Tools toolbox implemented in CONN to detect potential outlier scans identified from the observed global blood-oxygen-level-dependent (BOLD) signal and the amount of participant motion. We flagged acquisitions with framewise displacement greater than 0.9 mm or global BOLD signal changes greater than 5 S.D.s as potential outliers.

2.3.4 Graph theory analyses

We performed graph theory analyses using the default functional connectivity processing pipeline in the CONN toolbox. We first cleaned the preprocessed fMRI data using the CONN functional connectivity toolbox denoising pipeline. This included ART-based motion scrubbing, outlier volume removal, regression of white matter and cerebrospinal fluid signals using the component-based noise correction method (CompCor) strategy (34), band-pass temporal filtering ($f = 0.007$–0.1 Hz), and linear detrending. We derived the regions of interest (ROIs) used here from a freely available ROI atlas (35) which included 200 ROIs generated by a gradient-weighted Markov Random Field model that could parcelize neural regions representing neurobiologically meaningful features of brain organization using rs-fMRI data from 1,489 participants. We treated each ROI as a "node" within a whole-brain network. We extracted the BOLD time course for each ROI for each participant to create a graph adjacency matrix. First, for all pairs of ROIs, we created an ROI-to-ROI Correlation (RRC) matrix (γ) using an extracted time course. We then formed a graph adjacency matrix by thresholding the RRC matrix and retaining 15% of the strongest positive correlations; thus, in each participant graph, 15% of all possible edges were represented. These graphs were undirected in that the association between regions was bidirectional. From the resulting graphs, we computed a number of measures addressing topological properties of each ROI within the graph. Therefore, at the second level, we adapted the CONN toolbox's automated graph theory analysis algorithms[1] to explore associations between graph theoretic metrics and macronutrient intake using rs-fMRI from the 2nd wave, controlling for sex and SES using a false discovery rate (FDR)-corrected *p*-value threshold of $p < 0.05$ (all

1 For a detailed description, see https://web.conn-toolbox.org/fmri-methods/connectivity-measures/graphs-roi-level.

reported p-values are two-tailed). We performed the same analysis in boys and girls separately controlling for SES.

We measured the following graph theoretic metrics: *Betweenness Centrality*—a centrality measure of a ROI that acts as a bridge along the shortest path between two other ROIs; *Local Efficiency*— a measure of local integration or coherence, characterizing the degree of inter-connectedness among all nodes within a node neighboring sub-graph; *Eigenvector Centrality*—the influence of a ROI based on its connectedness with other high-scoring nodes in a network; *Degree and Cost* at each ROI—measures of network centrality, characterizing the degree of local connectedness of each ROI within a graph; *Average Path Distance*—a measure of node centrality within a network, characterizing the degree of global connectedness of each ROI within a graph; *Clustering Coefficient*— a measure of the degree to which nodes in a graph tend to cluster together; *Global Efficiency* at a node—a measure of this node centrality within the network, characterizing the degree of global connectedness of each ROI; *Eccentricity*—the maximum distance between an ROI and all other ROIs. Each of these statistics emphasizes a distinct aspect of the nodes' varying roles in organizing information processing across the brain [for a review of these graph theory metrics, please see (23)].

2.3.5 Associations across intelligence, macronutrient intake, and neural development

To investigate not only the effect of neural development and macronutrient intake on intelligence but also the potential moderating effect of macronutrient intake on the relationship between neural development and intelligence, we performed moderation analysis using R statistical software (v4.3.0; R Foundation for Statistical Computing, Vienna, Austria). First, regarding the ROIs that showed significant associations with nutrient intake at the 2nd wave, we calculated changes in ROI-specific graph theoretic metrics from the 2nd to the 3rd waves and used them as neural development. We then performed a multiple regression model in which we set FIQ as the dependent variable, and macronutrient intake, neural development, and an interaction term for neural development and macronutrient intake as explanatory variables. We included sex in this model as a potential confounder only when considering all participants, but not when considering boys only or girls only.

3 Results

We performed two studies: Study 1 aimed to examine the association between macronutrient intake and intelligence using large adolescent cohort data (TTC), and Study 2 aimed to detect the effect of nutrition intake on neural development using rs-fMRI data derived from a subset TTC.

3.1 Study 1: macronutrient intake and intelligence

Multiple linear regression was performed to examine associations between macronutrient intake and intelligence. Multiple linear regression showed a positive association between protein intake and FIQ [beta coefficient (β) = 0.55, p = 0.004], a negative association between total calorie intake and FIQ (β = −2.51, p = 0.046), and a positive association between SES and FIQ (β = 0.29, p < 0.001). Carbohydrate and fat intake were not significantly associated with FIQ (β = 1.57, p = 0.062; β = 0.61, p = 0.085). The measurement gap between FIQ and nutrition intake measures, difference in BMI-for-age at age 12 and 14, and sex were not significantly associated with FIQ (ps > 0.090).

VIF for carbohydrate, fat, protein, and total calorie intake were 1367.22, 242.36, 69.00, and 3007.77, respectively, indicating high correlation. To address this multicollinearity problem, we tested associations between FIQ and each macronutrient separately. However, we found no significant association between FIQ and carbohydrate, fat, or protein intake (β = −0.029, p = 0.233; β = 0.005, p = 0.845; β = 0.029, p = 0.216, respectively) (Table 3).

FIQ in boys was positively associated with protein intake (β = 0.068, p = 0.031), but not with carbohydrate (β < −0.001, p = 0.993) or fat intake (β = 0.036, p = 0.251) (Table 3).

FIQ in girls was negatively associated with carbohydrate intake (β = −0.076, p = 0.024), but not with protein (β = −0.029, p = 0.387) or fat intake (β = −0.040, p = 0.235) (Table 3).

3.2 Study 2: macronutrient intake and brain networks

Using rs-fMRI at the 2nd wave, associations between several graph theory metrics for ROIs and each macronutrient intake were explored controlling for sex and SES. In all participants, greater carbohydrate intake was associated with less local efficiency in the left triangular part of the inferior frontal gyrus ([x, y, z] = [−51, 23, 8], t = −5.49, $p_{FDR\text{-}corrected}$ = 0.00018) and with greater betweenness centrality in the left middle temporal gyrus ([x, y, z] = [−61, −43, −13], t = 4.75, $p_{FDR\text{-}corrected}$ = 0.0027). Greater fat intake was associated with less local efficiency in the left triangular part of the inferior frontal gyrus ([x, y, z] = [−51, 23, 8], t = −4.13, $p_{FDR\text{-}corrected}$ = 0.02290) and with greater betweenness centrality in the left middle temporal gyrus ([x, y, z] = [−61, −43, −13], t = 4.72, $p_{FDR\text{-}corrected}$ = 0.0029). Greater protein intake was associated with greater betweenness centrality in the left middle temporal gyrus ([x, y, z] = [−61, −43, −13], t = 5.10, $p_{FDR\text{-}corrected}$ = 0.00075) (Figure 2).

In boys, after statistically controlling for SES, greater carbohydrate intake was associated with less local efficiency in the left triangular part of the inferior frontal gyrus ([x, y, z] = [−51, 23, 8], t = −4.77, $p_{FDR\text{-}corrected}$ = 0.0063) and with greater betweenness centrality in the left middle temporal gyrus ([x, y, z] = [−61, −43, −13], t = 4.95, $p_{FDR\text{-}corrected}$ = 0.0037). Greater fat intake was associated with greater betweenness centrality in the left middle temporal gyrus ([x, y, z] = [−61, −43, −13], t = 4.15, $p_{FDR\text{-}corrected}$ = 0.0404) and with less eccentricity in the left posterior cingulate gyrus (brodmann area[BA] 23) ([x, y, z] = [−5, −29, 27], t = −4.44, $p_{FDR\text{-}corrected}$ = 0.0173) and the right precuneus ([x, y, z] = [11, −74, 25], t = −4.05, $p_{FDR\text{-}corrected}$ = 0.0270). Greater protein intake was associated with greater betweenness centrality in the left middle temporal gyrus ([x, y, z] = [−61, −43, −13], t = 4.71, $p_{FDR\text{-}corrected}$ = 0.0187).

In girls, after statistically controlling for SES, we found no significant associations between graph theory metrics and each macronutrient intake.

TABLE 3 Multiple linear regression models describing the association between Full Scale IQ scores at the 2nd wave and macronutrient intakes at the 3rd wave.

Dependent variable	Explanatory variables [beta coefficient (β), p-value]				
All participants					
Model 1					
FIQ	Carbohydrate intake	The measurement gap between FIQ and nutrition intake measures	Difference between BMI-for-age at age 12 and age 14	SES	sex
	$\beta = -0.029$, $p = 0.233$	$\beta = -0.007$, $p = 0.766$	$\beta = 0.003$, $p = 0.915$	$\beta = 0.292$, $p < 0.001$	$\beta = 0.095$, $p = 0.057$
Model 2					
FIQ	Fat intake	The measurement gap between FIQ and nutrition intake measures	Difference between BMI-for-age at age 12 and age 14	SES	sex
	$\beta = 0.005$, $p = 0.845$	$\beta = -0.006$, $p = 0.784$	$\beta = 0.001$, $p = 0.972$	$\beta = 0.292$, $p < 0.001$	$\beta = 0.116$, $p = 0.015$
Model 3					
FIQ	Protein intake	The measurement gap between FIQ and nutrition intake measures	Difference between BMI-for-age at age 12 and age 14	SES	sex
	$\beta = 0.029$, $p = 0.216$	$\beta = -0.005$, $p = 0.819$	$\beta < 0.001$, $p = 1.00$	$\beta = 0.291$, $p < 0.001$	$\beta = 0.130$, $p = 0.008$
Boys					
Model 1					
FIQ	Carbohydrate intake	The measurement gap between FIQ and nutrition intake measures	Difference between BMI-for-age at age 12 and age 14	SES	
	$\beta < -0.001$, $p = 0.993$	$\beta = 0.004$, $p = 0.895$	$\beta = 0.002$, $p = 0.960$	$\beta = 0.313$, $p < 0.001$	
Model 2					
FIQ	Fat intake	The measurement gap between FIQ and nutrition intake measures	Difference between BMI-for-age at age 12 and age 14	SES	
	$\beta = 0.036$, $p = 0.251$	$\beta = 0.005$, $p = 0.878$	$\beta < 0.001$, $p = 0.994$	$\beta = 0.312$, $p < 0.001$	
Model 3					
FIQ	Protein intake	The measurement gap between FIQ and nutrition intake measures	Difference between BMI-for-age at age 12 and age 14	SES	
	$\beta = 0.068$, $p = 0.031$	$\beta = 0.006$, $p = 0.848$	$\beta = -0.001$, $p = 0\ 0.973$	$\beta = 0.311$, $p < 0.001$	
Girls					
Model 1					
FIQ	Carbohydrate intake	The measurement gap between FIQ and nutrition intake measures	Difference between BMI-for-age at age 12 and age 14	SES	
	$\beta = -0.076$, $p = 0.024$	$\beta = -0.023$, $p = 0.503$	$\beta = 0.005$, $p = 0.888$	$\beta = 0.266$, $p < 0.001$	
Model 2					
FIQ	Fat intake	The measurement gap between FIQ and nutrition intake measures	Difference in BMI-for-age between age at 12 to 14	SES	
	$\beta = -0.040$, $p = 0.235$	$\beta = -0.022$, $p = 0.516$	$\beta = 0.001$, $p = 0.965$	$\beta = 0.267$, $p < 0.001$	
Model 3					
FIQ	Protein intake	The measurement gap between FIQ and nutrition intake measures	Difference between BMI-for-age at age 12 and age 14	SES	
	$\beta = -0.029$, $p = 0.387$	$\beta = -0.022$, $p = 0.522$	$\beta = 0.001$, $p = 0.971$	$\beta = 0.267$, $p < 0.001$	

BMI, Body mass index; FIQ, Full scale intelligence quotient; SES, Socioeconomic status.

3.3 Study 2: associations across macronutrient intake, neural development, and intelligence

Moderation analysis was conducted using rs-fMRI at waves 2 and 3 to examine the effects of neural development and macronutrient intake on intelligence and the potential moderating effect of macronutrient intake on the relationship between neural development and intelligence. In all participants, we found no significant moderation effect of carbohydrate intake on the relationship between intelligence and change in local efficiency in the left triangular part of the inferior frontal gyrus or change in betweenness centrality in the

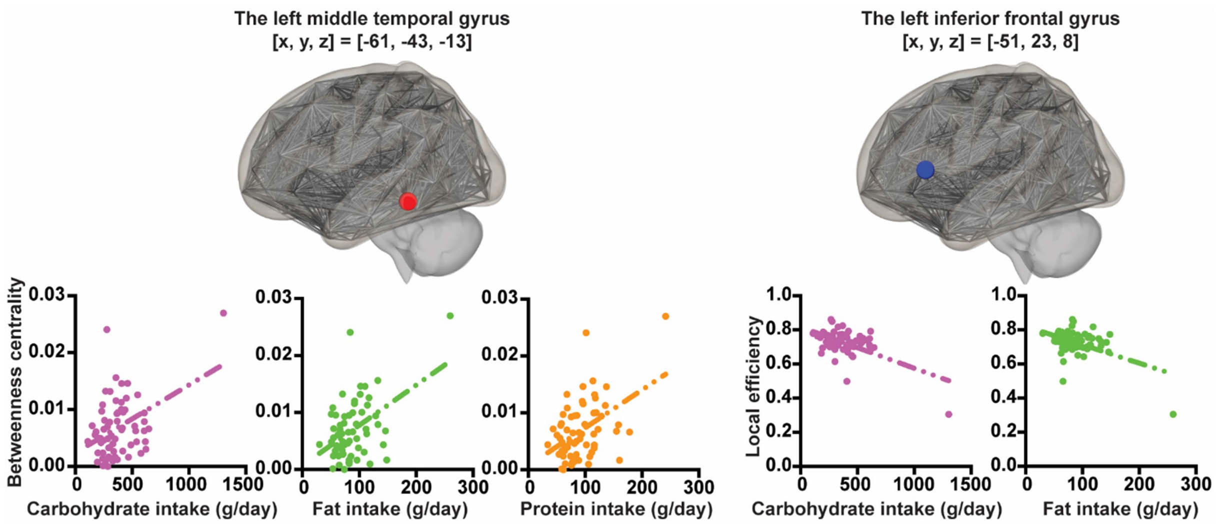

FIGURE 2
The associations between nutrient intake and graph theoretic metrics. The *X*-axis of the plot represents nutrition intake. The *Y*-axis of the plot represents graph theoretic metrics.

left middle temporal gyrus ($p=0.294$ and $p=0.1093$, respectively). Likewise, we found no significant moderation effect of fat intake on the relationship between intelligence and change in local efficiency in the left triangular part of the inferior frontal gyrus ($p=0.26$), but contrastingly, the moderation effect of fat intake on the relationship between intelligence and change in betweenness centrality in the left middle temporal gyrus was significant ($\beta=12.41$, $p=0.0457$) (Figure 3; Table 4). Similarly, the moderation effect of protein intake on the relationship between intelligence and change in betweenness centrality in the left middle temporal gyrus was significant ($\beta=12.12$, $p=0.0401$) (Figure 3; Table 4).

In boys, the moderation effect of carbohydrate intake on the relationship between intelligence and change in local efficiency in the left triangular part of the inferior frontal gyrus was not significant ($p=0.109$), whereas the moderation effect of carbohydrate intake on the relationship between intelligence and change in betweenness centrality in the left middle temporal gyrus was significant ($\beta=3.074$, $p=0.008$) (Figure 3; Table 4). The moderation effect of fat intake on the relationships between intelligence and change in local efficiency in the left triangular part of the inferior frontal gyrus, or change in eccentricity in the left posterior cingulate gyrus or in the right precuneus were not significant ($ps>0.261$), whereas the moderation effect of fat intake on the relationship between intelligence and change in betweenness centrality in the left middle temporal gyrus was significant ($\beta=16.72$, $p=0.008$) (Figure 3; Table 4). The moderation effect of protein intake on the relationship between intelligence and change in betweenness centrality in the left middle temporal gyrus was significant ($\beta=17.45$, $p=0.003$) (Figure 3; Table 4).

4 Discussion

Here we aimed to examine the impact of macronutrient intake on the association between intelligence and neural development in adolescents. Using a large adolescent cohort, we found that higher general intelligence was associated with increased protein intake in boys, and in girls, with lower carbohydrate intake. In addition, rs-fMRI data showed that lower carbohydrate and fat intake were associated with higher inter-connectedness in the left triangular part of the inferior frontal gyrus, and all macronutrient intake was positively correlated with greater centrality in the left temporal gyrus in all participants. Also, in boys, lower carbohydrate intake was associated with greater inter-connectedness in the left triangular part of the inferior frontal gyrus, whereas all macronutrient intake was positively correlated with greater centrality in the left temporal gyrus. Additionally, in boys, higher fat intake was associated with lower long-range connectivity in the left post cingulate gyrus and right precuneus. Moreover, in boys, but not girls, carbohydrate, fat, and protein intake moderated the relationship between general intelligence and neural development in the left middle temporal gyrus, indicating that the impact of neural development in the middle temporal gyrus on general intelligence differs depending on the level of macronutrient intake. Generally, in accordance with our hypothesis, protein intake was positively associated with higher general intelligence, while carbohydrate and fat intake were negatively associated with general intelligence and neural connectivity. These associations between macronutrient intake and intelligence or neural connectivity varied depending on sex.

In line with the hypothesis, higher protein intake was positively associated with greater intelligence in boys, partially replicating previous studies. Wang and colleagues reported a high protein dietary pattern in children aged 10–15 years was associated with greater mathematical ability after controlling for sex, age, nationality, household registration, school type, parental education level, family learning environment, annual household income, and family size (16). Another study showed a positive association in children aged 6–8 years between protein intake and higher cognitive function related to attention after controlling for age (17). Thus, protein intake during adolescence would facilitate general intelligence in boys.

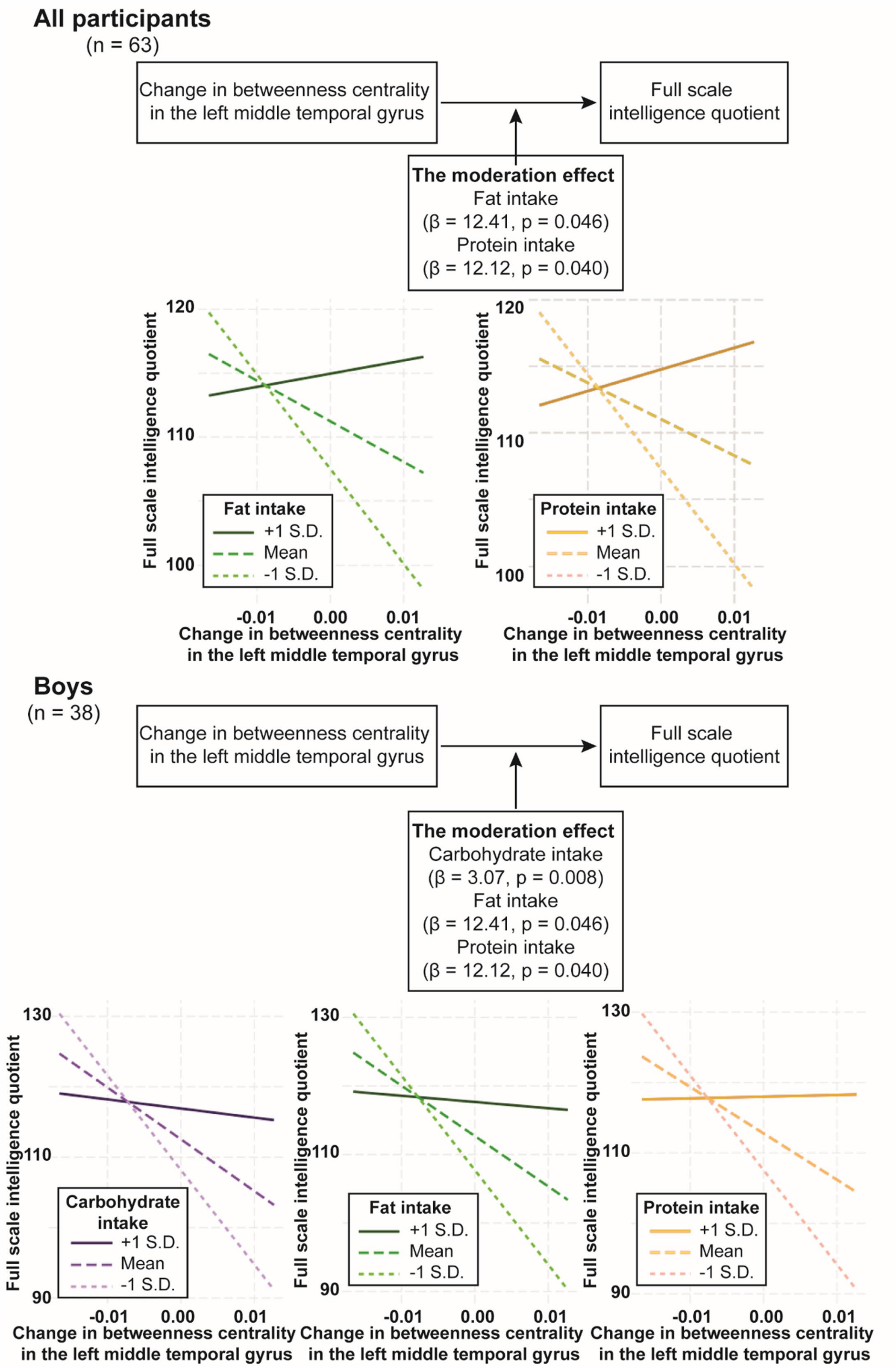

FIGURE 3
The moderation effect of nutrition intake on the relationship between full scale intelligence quotient change in betweenness centrality in the left middle temporal gyrus. The diagram depicts the moderation effect on the relationship between full scale intelligence quotient and change in betweenness centrality in the left middle temporal gyrus. The *X*-axis of the plot represents the independent variable, which is full scale intelligence quotient. The *Y*-axis of the plot represents the dependent variable, which is change in betweenness centrality in the left middle temporal gyrus. The lines on the plot represent different levels of the moderator variable. Each line's slope illustrates the relationship between full scale intelligence quotient and change in betweenness centrality in the left middle temporal gyrus at that specific level of the moderator variable. *β*, beta coefficient; S.D., standard deviation.

TABLE 4 Associations across macronutrient intake, neural development, and intelligence.

Dependent variable	Explanatory variables [beta coefficient (β), p-value]			
All participants				
FIQ	The moderation effect of carbohydrate intake	Carbohydrate intake	Change in betweenness centrality in the left middle temporal gyrus	Sex
	$\beta = 1.792$, $p = 0.109$	$\beta = 0.012$, $p = 0.393$	$\beta = -1{,}001$, $p = 0.089$	$\beta = 0.252$, $p = 0.954$
FIQ	The moderation effect of fat intake	Fat intake	Change in betweenness centrality in the left middle temporal gyrus	Sex
	$\beta = 12.41$, $p = 0.046$	$\beta = 0.110$, $p = 0.132$	$\beta = -1{,}406$, $p = 0.046$	$\beta = 0.482$, $p = 0.905$
FIQ	The moderation effect of protein intake	Protein intake	Change in betweenness centrality in the left middle temporal gyrus	Sex
	$\beta = 12.12$, $p = 0.040$	$\beta = 0.104$, $p = 0.094$	$\beta = -1{,}397$, $p = 0.055$	$\beta = 1.184$, $p = 0.776$
FIQ	The moderation effect of carbohydrate intake	Carbohydrate intake	Change in local efficiency in the left inferior frontal gyrus	Sex
	$\beta = -0.065$, $p = 0.294$	$\beta = 0.014$, $p = 0.397$	$\beta = 38.948$, $p = 0.421$	$\beta = 1.848$, $p = 0.664$
FIQ	The moderation effect of fat intake	Fat intake	Change in local efficiency in the left inferior frontal gyrus	Sex
	$\beta = -0.347$, $p = 0.261$	$\beta = 0.096$, $p = 0.212$	$\beta = 38.425$, $p = 0.447$	$\beta = 1.600$, $p = 0.683$
Boys				
FIQ	The moderation effect of carbohydrate intake	Carbohydrate intake	Change in betweenness centrality in the left middle temporal gyrus	
	$\beta = 3.074$, $p = 0.008$	$\beta = 0.02217$, $p = 0.118$	$\beta = -2068$, $p = 0.003$	
FIQ	The moderation effect of fat intake	Fat intake	Change in betweenness centrality in the left middle temporal gyrus	
	$\beta = 16.72$, $p = 0.008$	$\beta = 0.129$, $p = 0.085$	$\beta = -2{,}328$, $p = 0.003$	
FIQ	The moderation effect of protein intake	Protein intake	Change in betweenness centrality in the left middle temporal gyrus	
	$\beta = 17.45$, $p = 0.003$	$\beta = 0.133$, $p = 0.035$	$\beta = -2{,}449$, $p = 0.002$	
FIQ	The moderation effect of carbohydrate intake	Carbohydrate intake	Change in local efficiency in the left inferior frontal gyrus	
	$\beta = -0.110$, $p = 0.109$	$\beta = 0.030$, $p = 0.101$	$\beta = 63.176$, $p = 0.261$	
FIQ	The moderation effect of fat intake	Fat intake	Change in eccentricity in the left posterior cingulate gyrus	
	$\beta = -0.039$, $p = 0.877$	$\beta = 0.060$, $p = 0.349$	$\beta = 2.695$, $p = 0.904$	
FIQ	The moderation effect of fat intake	Fat intake	Change in eccentricity in the right precuneus	
	$\beta = -0.078$, $p = 0.621$	$\beta = 0.058$, $p = 0.346$	$\beta = 14.76$, $p = 0.387$	

FIQ, Full scale intelligence quotient.

As we expected, higher carbohydrate intake was negatively associated with general intelligence in girls. A previous study demonstrated that an unhealthy dietary pattern, such as the western diet, was connected with impaired brain development and lower IQ in early adolescents (15). In addition, another study with greater sample size ($n = 17{,}348$ school children) showed the connection between more frequent high-calorie food intake and children's weaker academic achievement (9). Therefore, in line with these results, our findings suggest that in girls, higher carbohydrate intake would be connected with lower intelligence.

Brain imaging data showed that, in all participants and boys, higher carbohydrate intake was negatively associated with lower local efficiency in the left triangular part of the inferior frontal gyrus. The triangular part of the inferior frontal gyrus acts as the hub that links the sensory/somatomotor network, the default mode network (DMN), and the dorsal and ventral attention network (36). This region is also involved in verbal and non-verbal context recognition (37), language processing (38), and unconscious information processing (39). Therefore, higher local efficiency in this region would indicate effective information processing—such as passing information through various brain networks, including the sensory/somatomotor network, DMN, and dorsal and ventral attention network—to facilitate an understanding of various contexts, which is a cognitive function implicated in intelligence (20, 40). The current study found a negative association between carbohydrate and fat intake and local efficiency in the triangular part of the inferior frontal gyrus. Additionally, carbohydrate intake was negatively associated with intelligence in girls. These findings suggest that excessive habitual carbohydrate intake may impair local efficiency in the triangular part of the inferior frontal gyrus and have a negative impact on intelligence in children, or at least girls.

We found that higher fat intake was linked to lower long-range connectivity in the left post cingulate gyrus and right precuneus in boys. The post cingulate gyrus (BA 23) is a part of the frontoparietal control network (38) and the DMN (41), constituting a core hub in the human connectome (42), with a relatively large-scale neural network (43), and is involved in various cognitive functions, including memory, spatial navigation, self-reflection and self-imagery, and decision making (44). The precuneus, involved in the visual field (38) and the DMN (45), has a large-scale neural network (46). Serving as a global hub, it is likely to be integrated in early adolescence (4). This region has a role in facilitating successful episodic memory retrieval (47). For instance, a previous fMRI study demonstrated the activation of this region during memory retrieval tasks (48). Further, the precuneus could be involved in executive functions, such as cognitive flexibility (49). Thus, long-range connectivity with the precuneus is related to various cognitive functions. Collectively, the post cingulate gyrus and precuneus have a long-range connectivity and play various roles in intelligence. Long-range functional connectivity—rather than short-range functional connectivity—could contribute to IQ (50). In addition, higher fat and sugar intake could be connected with attenuated IQ (12, 13, 15). As demonstrated by the current results, long-range connectivity in the post cingulate gyrus and precuneus were negatively associated with fat intake in boys; fat intake would negatively impact developing long-range functional connectivity in the post cingulate gyrus and precuneus and be connected with lower intelligence.

Brain imaging data also showed that, at the 2nd wave, greater intake of carbohydrate, fat, and protein was positively associated with greater betweenness centrality in the left middle temporal gyrus in all participants. Moreover, the moderation analysis revealed that the impact of neural development in betweenness centrality in the middle temporal gyrus on intelligence differs depending on the level of the moderation effect of carbohydrate, fat, and protein intake in boys, and the moderation effect of fat and protein intake in all participants. The middle temporal gyrus is a part of the frontoparietal control network (38) and involved in the regulation of perceptual attention (51), and also seems to be a part of the traditional sensory language area (52). Further, the region could be associated with the DMN (53) and creative ability (54). The middle temporal gyrus is thus involved in various cognitive functions related to intelligence. Given that the ROI with higher betweenness centrality more frequently lies on the shortest paths between other ROIs, greater betweenness centrality in the left middle temporal gyrus would suggest greater integrated functional connectivity within this region. The current results, consistent with previous studies (16, 17), suggest that higher protein intake in boys is associated with increased IQ. This supports our hypothesis that protein intake moderates connectivity integration in the middle temporal gyrus during development and promotes general intelligence.

The relationship between macronutrient intake and general intelligence, as well as the moderating effect of macronutrient intake on the relationship between neural development and general intelligence, are influenced by sex, which is consistent with our hypothesis. In a previous large-cohort study, sex differences in multiple cognitive functions appeared during the progression from early teens to late teens (5), and could be connected with brain maturation. Gray matter and white matter volumes in the frontal, temporal, and parietal regions show the effect of interaction between age and sex in adolescents (5). The functional and structural connectivity in the post cingulate gyrus also mediates sex differences in cognitive functions (6). Further, compared to boys, girls show earlier white matter development, and such sex difference influences cognitive performance (7). Thus, earlier brain maturation in girls is associated with their earlier cognitive development. Moreover, significant effects of sex, IQ, and age interactions on microstructures of the white matter were seen in frontoparietal areas (55). In the current study, only boys showed moderation effects of macronutrient intake on the association between IQ and neural development in the middle temporal gyrus, a part of the frontoparietal control network. These results suggest that neural development in boys at mid-teens is susceptible to macronutrient intake because of late maturation in the middle temporal gyrus. Given the link between structural and functional brain development and the surge of gonadal hormones (5), sex differences in neural and cognitive development may be attributed to gonadal hormones, in concert with which other factors—such as social and cultural factors (56)—may also contribute to such sex differences,. Further studies are needed to elucidate the cause of sex differences in neural and cognitive development.

Certain limitations should be considered in interpreting the current findings. First, although we included socioeconomic status, BMI-for-age, and sex as confounders, we did not include other potential confounders that may affect intelligence or neural development, such as environmental factors (10). Future studies should consider including these potential confounders to examine the effect of nutrition intake on intelligence or neural development in adolescents. Second, the number of participants for the imaging study and moderation analysis would not be adequate. Suitable sample sizes for functional neuroimaging studies using graph theory analysis have been debated, and a group size of approximately 20 was suggested as sufficient (57). Thus, in our study, the sample size for the rs-fMRI analysis could be acceptable. For the moderation analysis, we performed sample size estimation for the multiple regression analysis using G*Power (58) with the following parameters: number of predictors = 3, power = 0.80, $\alpha = 0.05$ and small ($f^2 = 0.02$), medium ($f^2 = 0.15$) or large ($f^2 = 0.35$) effect sizes. Estimated sample size for small, medium, and large effect sizes were 550, 77, and 36, respectively, indicating that the sample size for the moderation analysis may not be satisfactory, and the results of moderation analysis should be interpreted with caution. Third, we only had IQ data or dietary records at a single point in time. Previous studies have shown that the limited malleability of IQ by schooling and/or training (59) and IQ in early childhood are significant predictors of IQ through middle childhood into early adolescence (60). While other studies have suggested that the largest increase in human IQ is observed from 2 to 12 years, and by the age of 19–20, IQ reaches its maximum (61), and during this period, the effect of education on IQ development is observed (62). Thus, changes in IQ and nutrition intake over development should be considered in future studies to gain an in-depth understanding of associations across nutrient intake, neural development, and intelligence. Lastly, because of the age difference between the measurement of dietary habits (at age 14) and intelligence (at age 12), the current study could not examine the possibility that decreased inhibitory control, which is associated with lower general intelligence (63), could lead to increased consumption of an appetitive diet high in fat and sugar. Rodent studies consistently demonstrate that a diet high in fat and sugar can impair cognitive function, including memory (64) and spatial learning (65). Furthermore, considering the findings of a prior study that identified leaving the parental home and completing education as significant factors in altering dietary habits (29), it is unlikely that there will be a significant difference in the dietary habits of 12-year-old children (measured for intelligence) and 14-year-old children (measured for dietary habits) who typically reside at home and attend compulsory education in Japan. Thus, changing dietary habits between the ages of 12 and 14 is unlikely to have a significant impact on intelligence or cognitive function. However, to gain a complete understanding of the relationship between nutrition intake, intelligence, and neural development, further research is necessary.

In summary, our study examined the association between macronutrient intake, intelligence, and neural development in adolescents. Protein intake was positively associated with IQ in boys, whereas carbohydrate intake was negatively associated with IQ in girls in a large-cohort sample. Graph theory analysis of rs-fMRI data derived from a subsample of the large-cohort sample demonstrated that macronutrient intake was positively correlated with neural integration in the middle temporal gyrus in boys, but not in girls. Moreover, the impact of neural integration during development in the middle temporal gyrus on general intelligence differed depending on the level of macronutrient intake in boys. These results suggest that macronutrient intake in early adolescents influences neural development related to intelligence, and such influence of nutrition intake differs between boys and girls. Appropriate nutrition intake is therefore a key factor in healthy neural and cognitive development.

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 Tokyo Metropolitan Institute of Medical Science (approval number: 12–35); The University of Tokyo (10057); and SOKENDAI (The Graduate University for Advanced Studies; 2012002). 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.

Author contributions

YN: Conceptualization, Formal analysis, Investigation, Project administration, Supervision, Writing – original draft, Writing – review & editing. SY: Data curation, Funding acquisition, Writing – review & editing. NO: Data curation, Funding acquisition, Writing – review & editing. SA: Data curation, Funding acquisition, Writing – review & editing. AN: Data curation, Funding acquisition, Writing – review & editing. KK: Data curation, Funding acquisition, Writing – review & editing. SK: Data curation, Funding acquisition, 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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Central amygdala is related to the reduction of aggressive behavior by monosodium glutamate ingestion during the period of development in an ADHD model rat

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Introduction: Monosodium glutamate (MSG), an umami substance, stimulates the gut-brain axis communication via gut umami receptors and the subsequent vagus nerves. However, the brain mechanism underlying the effect of MSG ingestion during the developmental period on aggression has not yet been clarified. We first tried to establish new experimental conditions to be more appropriate for detailed analysis of the brain, and then investigated the effects of MSG ingestion on aggressive behavior during the developmental stage of an ADHD rat model.

Methods: Long-Evans, WKY/Izm, SHR/Izm, and SHR-SP/Ezo were individually housed from postnatal day 25 for 5 weeks. Post-weaning social isolation (PWSI) was given to escalate aggressive behavior. The resident-intruder test, that is conducted during the subjective night, was used for a detailed analysis of aggression, including the frequency, duration, and latency of anogenital sniffing, aggressive grooming, and attack behavior. Immunohistochemistry of c-Fos expression was conducted in all strains to predict potential aggression-related brain areas. Finally, the most aggressive strain, SHR/Izm, a known model of attention-deficit hyperactivity disorder (ADHD), was used to investigate the effect of MSG ingestion (60 mM solution) on aggression, followed by c-Fos immunostaining in aggression-related areas. Bilateral subdiaphragmatic vagotomy was performed to verify the importance of gut-brain interactions in the effect of MSG.

Results: The resident intruder test revealed that SHR/Izm rats were the most aggressive among the four strains for all aggression parameters tested. SHR/Izm rats also showed the highest number of c-Fos + cells in aggression-related brain areas, including the central amygdala (CeA). MSG ingestion significantly decreased the frequency and duration of aggressive grooming and attack behavior and increased the latency of attack behavior. Furthermore, MSG administration successfully increased c-Fos positive cell number in the intermediate nucleus of the solitary tract (iNTS), a terminal of the gastrointestinal sensory afferent fiber of the vagus nerve, and modulated c-Fos positive cells in the CeA. Interestingly, vagotomy diminished the MSG effects on aggression and c-Fos expression in the iNTS and CeA.

Conclusion: MSG ingestion decreased PWSI-induced aggression in SHR/Izm, which was mediated by the vagus nerve related to the stimulation of iNTS and modulation of CeA activity.

KEYWORDS

umami, c-Fos, aggression, resident-intruder test, gut-brain axis, vagus nerve, social isolation

1 Introduction

Developmental events, including the interplay of genetic, biological, environmental, and individual factors, can profoundly impact long-term emotional and behavioral responses (1–4). The long-term consequences of early life experiences play a crucial role in the onset of psychopathologies later in life (5–7). External environmental stimuli during development can affect the formation of emotions such as anxiety, antisocial behavior, and aggression (3, 8, 9). Aggression, a key symptom in various psychological disorders like mood disorders, personality disorders, substance abuse, schizophrenia, and autism, is often linked to early life environmental stressors such as social rejection or isolation in humans and other species (6, 10, 11). The impact of social isolation on aggressive behavior is contingent on the stage of development in rodents (10). Aggression levels increased in adult animals that underwent post-weaning social isolation (PWSI) (12, 13). PWSI disrupts the preference for social stimulus in adulthood and promotes the escalation of aggressive behaviors (7, 13–17).

The effect of social isolation stress during development on aggressive behavior largely depends on the strain, sex, and species of the animal model (10, 18, 19). The inbred juvenile spontaneously hypertensive rats (SHR) that exhibit hyperactivity, inattention, impulsivity, and learning deficits in various behavioral paradigms have been widely used as behavioral models of attention-deficit hyperactivity disorder (ADHD) (20, 21). Although stroke-prone SHR (SHR-SP) have been extensively studied as an ADHD model, no studies have reported which strain exhibits more aggressive behavior (22, 23). Impulsive aggression is a clinically distinct and common behavior in ADHD and autism, with 54% of ADHD patients showing clinical aggression (24). Individuals with ADHD tend to be more vulnerable to the impact of social deprivation (25, 26). Due to impulsivity and emotional dysregulation, aggression can be a challenging and complex issue for individuals with ADHD (25–29).

Food and nutrition are potential environmental modifications that may have an impact on behavior (29–32). The phenomenon by which dietary components stimulate the gastrointestinal tract, impact the brain, and ultimately determine behavior has recently gained scientific attention (33, 34). The gut-brain axis is known to play an important role in regulating brain function, and as a result, influences psychological and emotional stability (35, 36). L-glutamate, a savory umami taste substance, has the potential to play a role in gut-brain axis communication via activation of taste receptors and subsequent vagus nerve (37–40). Our previous data in SHR showed that monosodium glutamate (MSG) ingestion successfully decreased strong aggressive behavior in a social interaction test (41). This effect was mediated via the vagus nerve, as proven by diminishing the effect of decreasing aggression by MSG after vagotomy (41). However, the data appeared to vary depending on the experimental conditions, despite showing a significant difference between groups.

Therefore, in this study, we initially aimed to establish improved experimental conditions to investigate the detailed brain mechanism of reducing aggression due to MSG ingestion. Specifically, we substituted the social interaction test with the resident-intruder test and observed aggressive behavior under dim red light during subjective nights to align with the behaviors of nocturnal animals. Prior to this, we compared aggression levels in different rat strains, including SHR/Izm, SHR-SP/Ezo, WKY/Izm, a genetic control rat, and Long-Evans, a strain commonly used for studying aggression (20–22, 42, 43), to confirm the proper model of aggression with a neuropathological background of ADHD. Furthermore, we analyzed aggression-related brain areas in these strains by comparing neuronal activation based on c-Fos expression (44, 45).

Finally, the effect of MSG on aggression was investigated using the resident-intruder test in the most aggressive strain SHR/Izm. We validated the role of vagus nerve activation following MSG ingestion by conducting vagotomy and assessing neuronal activity in the nucleus of the solitary tract (NTS), which is the terminus of the vagus nerve's gastrointestinal sensory afferent fibers (46). Since the brain mechanism underlying the effect of MSG ingestion has not been clarified yet, we focused on the alteration of neural activity in aggression-related areas of the brain, including the amygdala. The amygdala has been known to play a role in aggressive behavior and emotional instability in ADHD (47–50). The direct and indirect neuronal projections between the NTS and central amygdala (CeA) could lead to the hypothesis that NTS activation by MSG via the vagus nerve may potentially modulate neuronal activity in the CeA, ultimately affecting aggression (51–54).

2 Materials and methods

2.1 Animals

For experiments in strain-difference, male rats of four different strains (SHR/Izm, and SHR-SP/Ezo, WKY/Izm, and Long-Evans; $n = 6$/each strain) were purchased from Japan SLC Inc. (Hamamatsu, Japan). SHR/Izm represents an animal model of ADHD characterized by hyperactivity and inattention without anxiety-related impulsive behavior when compared to WKY (Wistar-Kyoto) rats as a genetic control (55, 56). SHR-SP is a sub-strain of SHR that exhibits more inattention and impulsivity than SHR (22, 57). Long-Evans was included for comparison, as it is a commonly used laboratory rat in studies aggression related to social isolation and intermale aggression (42, 43, 58, 59).

The most aggressive strain, SHR/Izm, was used to investigate the effects of MSG ingestion (60 mM solution) on aggression. A total of 24 male SHR/Izm were assigned to two groups: MSG group ($n = 12$) and control group ($n = 12$). Male Wistar rats ($n = 12$) were obtained from Japan SLC Inc. and used as intruders to induce aggression.

The rats were housed under temperature-controlled conditions (23-25°C, average humidity 50%) with free access to standard chow (MFG; Oriental Yeast Co. Ltd.) and water on a 12-h light/dark cycle (lights on at 22:00 and off at 10:00). To escalate aggression, animals were housed individually from P25 to P60 for 5 weeks in standard cages ($40 \times 23 \times 18$ cm) under conditions of low wood shaving bedding and relatively high illumination in the light phase. After 5 weeks of isolation, behavioral assessments, including the open-field test and resident-intruder test, were conducted in the dark phase at 13:00–16:00 to get more stable results. Intruder rats were group-housed (2–3 males per cage) in standard cages on a 12 h light/dark cycle (lights on at 08.00 and off at 20:00). MSG 60 mM solution (MP Biomedicals, United States, 101800) or distilled water was administered via drinking bottles *ad libitum* until behavioral tests were completed and the brains were obtained for c-Fos immunostaining. The drinking bottle was changed three times per week. Body weight and drinking volume were measured three times weekly.

To confirm the MSG action on c-Fos expression in the brain without behavioral effect of the resident intruder test, 18 male SHR/Izm at 8 weeks-old that were group-housed (2–3 males per cage) in standard cages on a 12 h light/dark cycle (lights on at 08.00 and off at 20:00) were assigned to three groups (60 mM MSG group, $n = 6$; 180 mM MSG group, $n = 6$; and control group, $n = 6$) and then MSG was administered by gavage one shot.

Additional experiments were conducted for 5 weeks to confirm the effect of MSG ingestion without behavioral effects and to clarify the effects of PWSI. Twelve male SHR/Izm rats were assigned to three groups: control group-housed group ($n = 4$), control PWSI group ($n = 4$), and MSG PWSI group ($n = 4$). MSG 60 mM solution or distilled water was administered *ad libitum* via drinking bottles.

Every effort was made to minimize suffering and the number of animals used. All experimental procedures were approved by the Committee on Animal Experimentation of Nagoya City University Medical School, and were in accordance with the animal care guidelines of Nagoya City University.

2.2 Behavioral test

2.2.1 Open field test

The rat at P60 was allowed to move freely for 10 min in a black circular arena (60 cm diameter × 50 cm height) in the dark phase (13:00–16:00) under red dim light (~2 lux) and recorded using an overhead camera for the following analysis (60). The recorded behavior was analyzed using an automated tracking system Smart software (Bio Research Center Inc., Nagoya, Japan) with the following parameters: (1) total distance moved, (2) duration of inactivity, (3) frequency of entrance into the center area, and (4) time spent in the center area (61, 62).

2.2.2 Resident-intruder test

Aggressive behavior in several strains of rats, including Long-Evans, WKY/Izm, SHR/Izm, and SHR-SP/Ezo, was induced in the resident's home cage by the intruder. The intruder is a male Wistar rat with a slightly smaller body weight (approximately 20 grams) compared to resident. Wood shaving bedding in the resident cage was kept for 1 week before the resident-intruder test. This test was performed three times in three consecutive days in the dark phase (13:00–16:00) under red dim light using the same resident and intruder pair.

Animal behavior was video-recorded using an overhead camera for 10 min and analyzed by measuring the frequency, duration, and latency of anogenital sniffing (weak aggression), aggressive grooming (moderate aggression), and attack behavior (strong aggression). Aggressive grooming is characterized by a lateral threat, upright posture, rearing or pouncing, and chasing. Attack behavior is defined as biting, clinch attack, and keeping down (63). Manual behavioral annotation and tracking were performed using Smart software (Bio Research Center Inc., Nagoya, Japan).

2.3 Immunohistochemistry of c-Fos

To determine the areas of the brain that are related to aggression and those that are affected by the administration of MSG, IHC of c-Fos was conducted to assess neuronal activity in the brain. Ninety minutes after the resident intruder test, the rats were deeply anesthetized with pentobarbital sodium (100 mg/kg, i.p.; Tokyo Kasei, Tokyo, Japan) and perfused transcardially with 0.1 M phosphate-buffered saline (PBS, pH 7.4) followed by 4% (w/v) paraformaldehyde (PFA, Sigma-Aldrich., St. Louis, United States) in PBS. The brains were post-fixed in 4% PFA overnight at 4°C, followed by 30% (w/v) sucrose until submerged. The brains were embedded in O.C.T. compound (Tissue-Tek, Sakura Finetek Japan Co., Ltd.) and frozen. Serial coronal sections (40 µm) were prepared using a cryostat (Leica CM 1520, Japan) and collected in a cryoprotectant (anti-frozen solution) containing 25% Ethylene Glycol and 25% Glycerol in PB before histological analysis.

For immunohistochemistry, the sections were first washed with PBS for 5 min three times and incubated with 0.6% (v/v) H_2O_2 (Wako., Tokyo Japan) dissolved in PBS + 0.1% (v/v) Triton X-100 (Nacalai Tesque, Inc., Kyoto, Japan) for 30 min to inactivate endogenous peroxidase, and then incubated in a blocking solution (10% horse serum in PBS containing 0.3% Triton X-100) for 60 min following washing (PBS-T: PBS + 0.3% Triton X-100) for 5 min three times.

The sections were incubated with mouse monoclonal anti-c-Fos antibody (1:1000; EnCor Biotechnology Inc., Florida, MCA 2H2, lot.030123). After washing with 1% horse serum in PBS-T three times, the sections were incubated for 120 min in 4.5 µL/mL of biotinylated anti-mouse IgG antibody (Vector Laboratories, Inc., California, BA-2000, lot.2B0622) diluted with 1% horse serum in PBS-T at room temperature. The sections were then washed three times in PBS-T, followed by incubation in 9 µL/mL of avidin-biotin complex (Vectastain ABC kit; Vector Laboratory, Inc., United States, PK4000, lot. 2 J1116) in PBS-T for 60 min. The sections were visualized with 0.25 mg/mL diaminobenzidine dissolved in PBS containing 0.009% H2O2 for 5–10 min at room temperature. The sections were mounted on gelatin-coated slides, air-dried, and gradually dehydrated using 50 to 100% ethanol. The brain sections were embedded in a cover slide.

Microscopic images were obtained using an Olympus AX70 microscope integrated with the U-PHOTO Universal Photo System. C-Fos positive cells (appearing as round shape and dark brown color) were manually counted in each region of interest (Paxinos and Watson Brain Map) using ImageJ software. The number of positive c-Fos cells was counted on both the left and right sides from four sections for each brain area in each rat.

2.4 Subdiaphragmatic vagotomy

Vagotomy was carried out at the sub-diaphragmatic level using SHR/Izm at P24, according to our previous report with some modifications (41). Briefly, after overnight food restrictions, the rats were anesthetized with an intraperitoneal injection of a 2 mL/kg mixture of medetomidine (0.185 mg/mL; Fujita Pharmaceutical Co., Ltd., Tokyo Japan), midazolam (1 mg/mL; Sando Co., Ltd., Tokyo Japan), and vetorphale (1.25 mg/mL: Meiji Animal Health Co. Ltd., Tokyo Japan). After a midline incision of the abdomen, the left lobes of the liver were moved aside and covered with saline gauze, and the stomach and lower esophagus were exteriorized from the peritoneal cavity and kept wet with saline. The dorsal and ventral trunks of the vagus nerve on the lower esophagus were cut at the subdiaphragmatic level using electrocauterization under a microscope. Subsequently, the organs were placed in the appropriate position, and the muscle and skin were tightly sutured. After the surgery, atipamezole hydrochloride (Orion Pharma, Expoo, Finland) was administered after completion of all surgical procedures at a dose rate of 0.4 mg/kg (i.p.). The rats were then returned to their home cages. Five weeks of individual housing and MSG administration via drinking bottles were performed before the behavioral test.

2.5 Statistical analysis

GraphPad Prism 9.0.0 software (GraphPad Software Inc.) was used for the statistical analysis. The resident-intruder test was analyzed using non-parametric analysis by Mann–Whitney or multiple comparisons Kruskal-Wallis followed by Dunn's test. The open field test and immunohistochemistry data were analyzed using parametric analysis by unpaired t-test or one-way ANOVA, followed by Tukey's multiple comparison test as a post-hoc test. A non-parametric analysis was conducted if the dataset did not follow a normal distribution. Differences were considered statistically significant at $p < 0.05$.

3 Results

3.1 Isolation-induced aggressive behavior in strain-dependent rats

We previously reported that MSG ingestion, mediated by the vagus nerve, reduced aggressive behavior in the social interaction test (41). However, the data obtained from the social interaction test showed large variability, although significant behavioral differences were observed between the groups. Therefore, we first attempted to establish new experimental conditions that are more appropriate for a detailed analysis of the effect of MSG on the brain. Specifically, we substituted a social interaction test with a resident-intruder test conducted over three consecutive days, and observed aggressive behavior under dim red light during the subjective night, mimicking the conditions of nocturnal animals. We found that by providing relatively high illumination during the subjective day and conducting the resident-intruder test under dim red light during the subjective night, PWSI for 5 weeks from P25-P60 consistently and stably induced weak, moderate, and strong aggressive behavior (Figure 1).

After setting more appropriate conditions, we next compared aggressive behaviors across four different rat strains using the resident-intruder test: Long-Evans, WKY/Izm, SHR/Izm, and SHR-SP/Ezo (Figure 1). Although all strains exhibited anogenital sniffing (weak aggression) on the first day of the resident-intruder test (Figures 1A–C), we noticed an apparent enhancement in aggressive grooming (moderate aggression; Figures 1D–F) and attack (strong aggression; Figures 1G–I) day by day, with no difference between the second and third days. Among the four strains, Long-Evans was the calmest, exhibiting mild aggressive grooming with the lowest frequency, shortest duration, and longest latency (Figures 1D–F), without any attack behavior (Figures 1G–I). Conversely, SHR/Izm was the most aggressive strain, showing the highest frequency, longest duration, and shortest latency of attacks, especially on the second day (Figures 1G–I).

Thus, we revealed that only SHR/Izm showed significant differences from Long-Evans in all aggression parameters, including the frequency and duration of weak, moderate, and strong aggressive behavior.

3.2 Increasing c-Fos positive cells in the brain area associated with aggressive behavior

Some brain areas are known to be related to aggressive behavior (18, 19, 64, 65). To investigate which brain areas are associated with strain differences in escalated aggression, IHC of c-Fos was performed in three different rat strains: Long-Evans, WKY/Izm, and SHR/Izm. The focus was on aggression-related brain areas such as the prefrontal cortex (PFC), central amygdala (CeA), lateral hypothalamus (LH), locus coeruleus (LC), periaqueductal gray (PAG), and dorsal raphe nucleus (DRN; Figure 2).

A higher number of c-Fos-positive cells was observed in the brains of WKY/Izm and SHR/Izm compared to the less aggressive strain rat, Long-Evans, especially in the PFC (Figures 2A,C), CeA (Figures 2B,D), LH (Figure 2E), and LC (Figure 2F). It is noted that the difference in c-Fos positive cells between Long-Evans and SHR/Izm was considerably large in the PFC ($p = 0.0042$), CeA ($p = 0.0074$), and LH ($p = 0.0032$; Figures 2C–E). However, no significant difference in c-Fos positive cells was detected in the PAG (Figure 2G) or DRN (Figure 2H) across the three rat strains.

3.3 Oral ingestion of MSG decreased isolation-induced aggression in SHR/Izm rats

To confirm the effect of MSG in reducing aggression using our new experimental conditions, SHR/Izm rats were chosen based on previous data indicating that this strain is the most aggressive (Figure 1). Since the aggression on the second day of the resident-intruder test was obvious and consistent, we used data from the

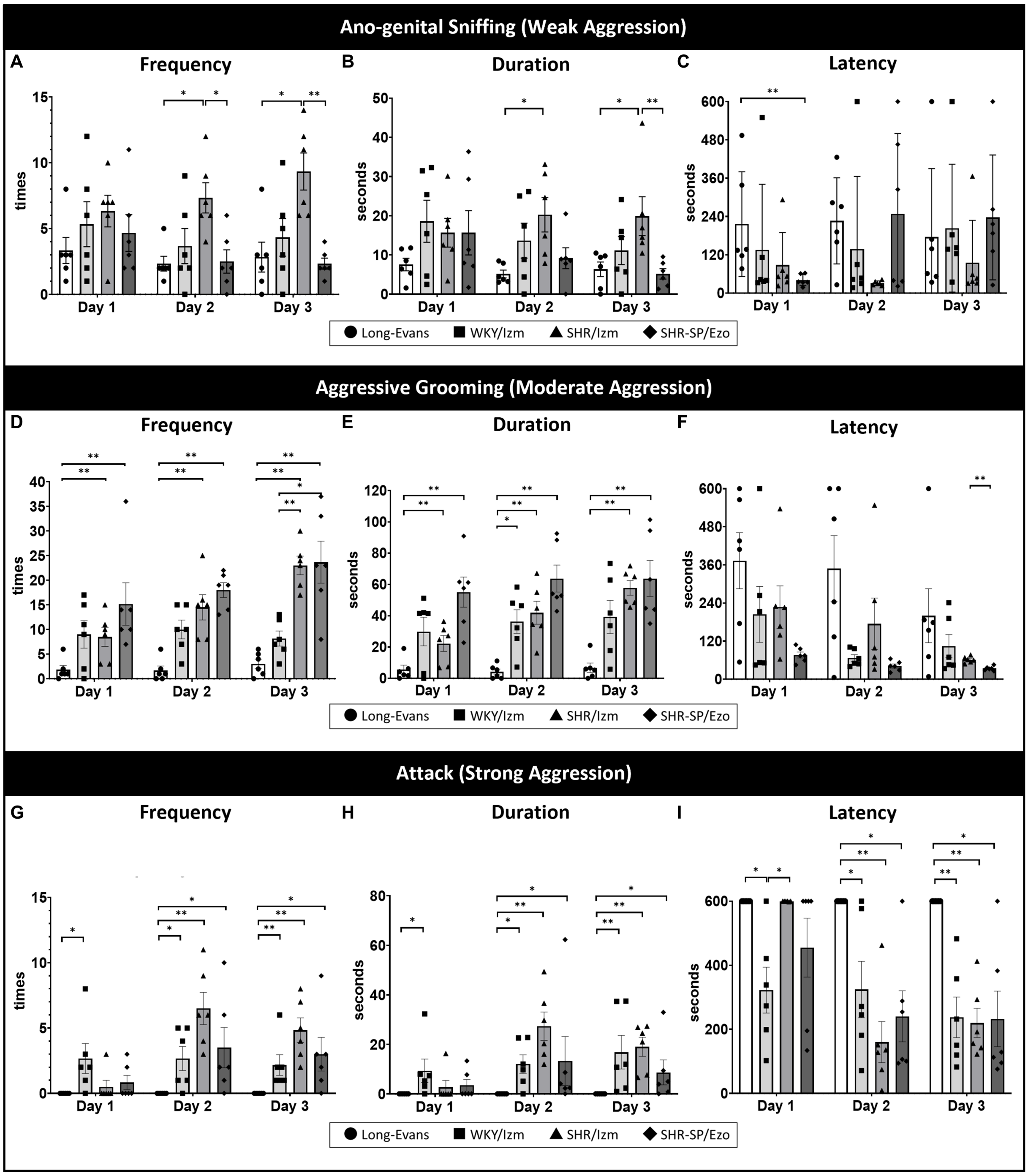

FIGURE 1
Isolation-induced aggressive behavior in strain-dependent rats was shown using a resident intruder test. The resident-intruder test was conducted to compare aggressive behavior among four different strains of rats: Long-Evans ($n = 6$), WKY/Izm ($n = 6$), SHR/Izm ($n = 6$), and SHR-SP/Ezo ($n = 6$) over three consecutive days. **(A–C)** SHR/Izm rats showed the highest frequency **(A)** and duration **(B)** of anogenital-sniffing (mild aggression) compared to other strains on days 2 and 3, even though there was no significant difference in the latency of anogenital-sniffing **(C)**. **(D–F)** High frequency **(D)** and long duration **(E)** of aggressive grooming (moderate aggression) were demonstrated by WKY/Izm, SHR/Izm, and SHR-SP/Ezo, whereas no difference in the latency of aggressive grooming was observed **(F)**. **(G,H)** SHR/Izm rats were the most aggressive strains, with the highest frequency **(G)**, longest duration **(H)**, and shortest attack behavior (strong aggression) **(I)**, showing a consistent significant difference compared with Long-Evans rats on three consecutive days of the resident-intruder test. Each bar represents the mean ± SEM ($n = 6$/each group); $*p < 0.05$, $**p < 0.01$, statistical analysis was performed using multiple comparisons (non-parametric test) using the Kruskal-Wallis test followed by Dunn's test.

second day to compare the effect of MSG on aggression (Figure 3). MSG ingestion significantly decreased the frequency and duration of aggressive grooming (moderate aggression) and attack behavior (strong aggression; Figures 3A,B), and increased the latency of attack behavior (Figure 3C) compared to the control group that received drinking water without MSG.

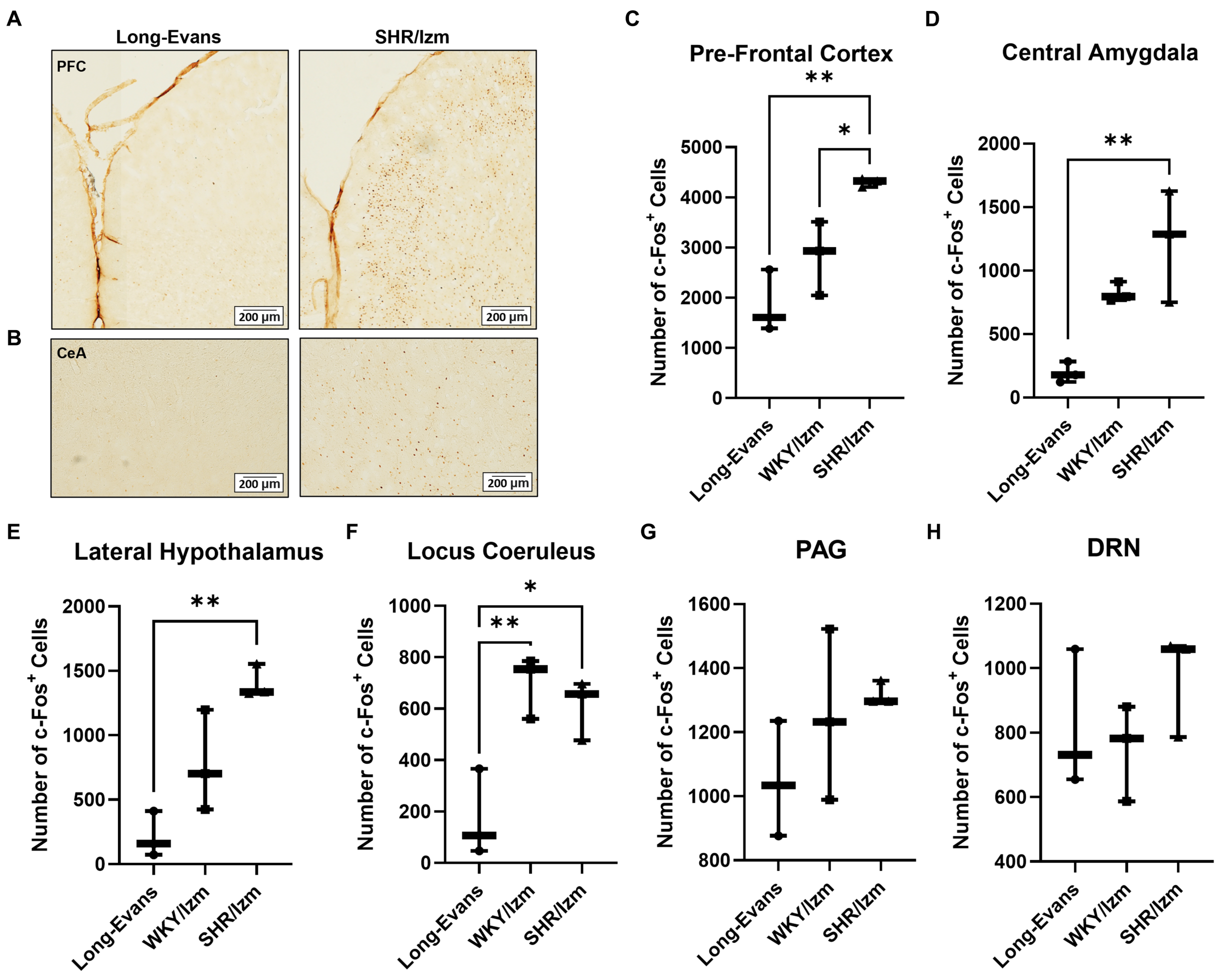

FIGURE 2
Increasing the number of c-Fos + cells in the brain areas associated with aggressive behavior. **(A,B)** Overview of c-Fos immunoreactivity in coronal sections of Pre-Frontal Cortex **(A)** and Central Amygdala **(B)** from Long-Evans rats and SHR/Izm rats as a result of IHC using anti c-Fos antibody. **(C–H)** Number of c-Fos + cells were counted from several areas of the brain related to aggression, including Pre-Frontal Cortex (PFC) **(C)**, Central Amygdala (CeA) **(D)**, Lateral Hypothalamus (LH) **(E)** Locus Coeruleus (LC) **(F)**, Peri-aquaductal Grey (PAG) **(G)**, and Dorso-Raphe Nuclei (DRN) **(H)** of Long-Evans (n = 3), WKY/Izm (n = 3) and SHR/Izm rats (n = 3). Increased c-Fos + cells were shown by SHR/Izm, especially significantly different compared with Long-Evans in the PFC, CeA, LH, and LC. Each bar represents the mean ± SEM (n = 6); *p < 0.05, **p < 0.01, statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test.

To confirm the effect of MSG on anxiety-like behavior under our new experimental conditions, an open field test was performed after 5 weeks of PWSI. No significant differences in the total distance traveled (Figure 4A), total number of entrances into the center area (Figure 4B), time spent in the center area (Figure 4C), and total duration of inactivity (Figure 4D) were observed between the MSG and control groups.

3.4 MSG administration increase c-Fos positive cells in the intermediate nucleus of solitary tract

Our previous data showed that reduced aggression in the social interaction test was mediated by the vagus nerve (41). To confirm that the effect of MSG is related to the activation of the vagus nerve, which is connected to umami receptor stimulation in the gut, we performed c-Fos immunostaining in the nucleus of the solitary tract (NTS; Figure 5). We focused on both the intermediate part of the NTS (iNTS) as a terminal of the gastrointestinal sensory afferent fiber of the vagus nerve, and the rostral part of the NTS (rNTS) as a terminal of the tongue sensory afferent fiber of the glossopharyngeal nerve (46, 66).

Even though IHC staining was conducted after performing the resident intruder test without long-term fasting (Figure 5A), MSG ingestion significantly increased the number of c-Fos + cells in the iNTS (p = 0.0094; Figures 5B,D–F), whereas no difference was observed in the rNTS between both groups (Figure 5C).

3.5 The effect of MSG ingestion on c-Fos positive cells in the brain area associated with aggressive behavior

We further investigated c-Fos expression patterns in aggression-related brain areas, such as the PFC, CeA, and LH, after MSG ingestion (Figure 6). The number of c-Fos + cells in the PFC and LH was comparable between the MSG-treated (n = 4) and control groups (n = 4; Figures 6A,B). However, the number of c-Fos + cells in the CeA was significantly lower in the MSG group than that in the control group (p = 0.0084; Figures 6C,D).

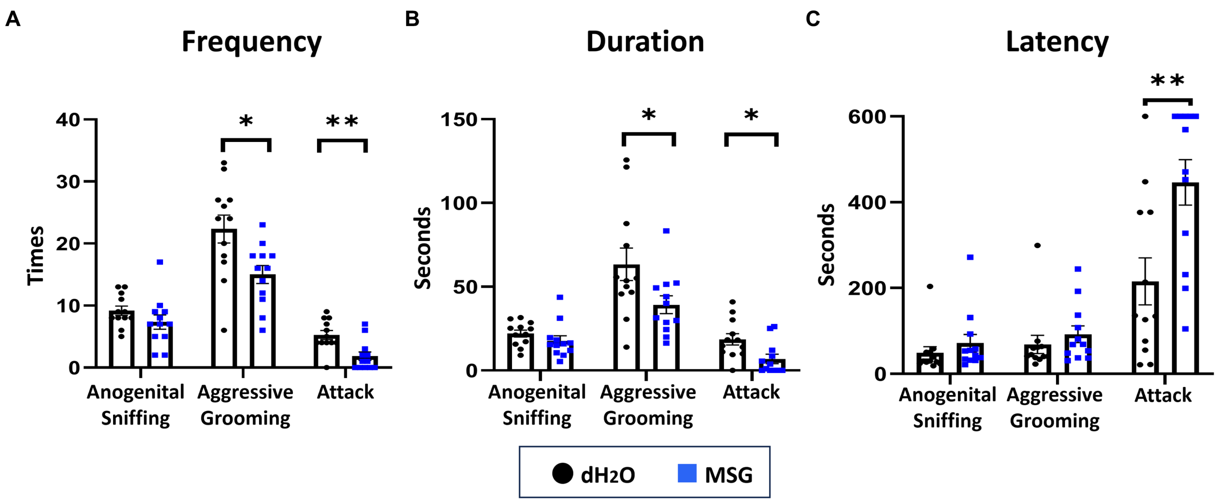

FIGURE 3
Oral ingestion of MSG decreased isolation-induced aggressive behavior in SHR/Izm rats was shown by the resident intruder test. The results of the resident intruder test showed that MSG ingestion significantly decreased the frequency of aggressive grooming and attack behavior **(A)**, decreased the duration of aggressive grooming and attack behavior **(B)**, and increased the latency of attack behavior **(C)** compared with the control group. Each bar represents the mean ± SEM ($n = 12$); *$p < 0.05$, **$p < 0.01$, statistical analysis was performed using Mann–Whitney test.

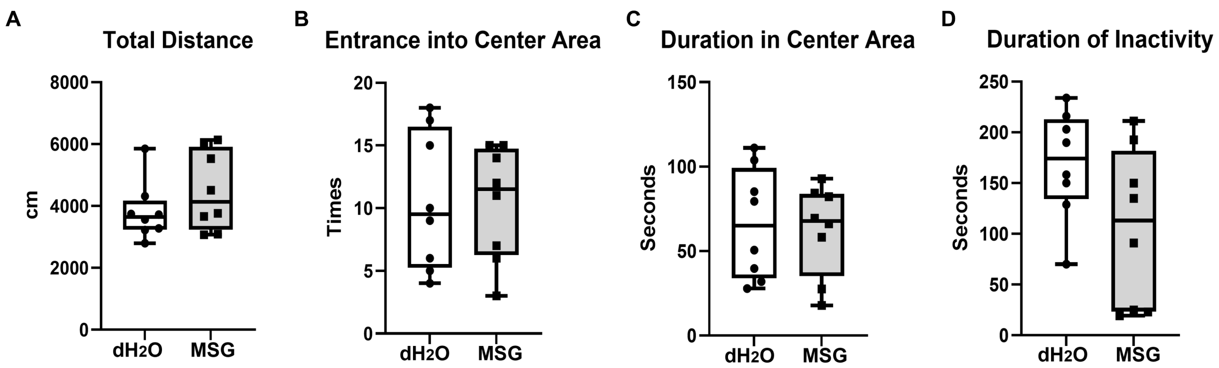

FIGURE 4
Oral ingestion of MSG did not affect the anxiety-like behaviors analyzed by open field test. The results of the open field test showed no difference between the MSG and control groups in the total distance traveled **(A)**, number of entrances into the center area **(B)**, time spent in the center area **(C)**, and total duration of inactivity **(D)**. Each bar represents the mean ± SEM ($n = 8$); statistical analysis was performed using unpaired t-test.

3.6 Intragastric administration of MSG increase c-Fos positive cells in the intermediate nucleus of solitary tract and central amygdala

To confirm the direct action of MSG on c-Fos expression (without the effects of the resident intruder test and PWSI), we conducted another experiment without prior individual housing and resident intruder tests using male SHR/Izm at 8 weeks-old (Figure 7A). In this protocol, rats were directly administered MSG into the stomach after overnight fasting. Ninety minutes after MSG administration, c-Fos expression in the iNTS and rNTS was investigated.

We found that intragastric administration of 180 mM MSG resulted in a significant increase in c-Fos positive cells in the iNTS (Figures 7B,D). However, no positive cells were detected in the rNTS of the MSG-treated groups (Figure 7C), as in the control group.

Interestingly, in contrast to long-term MSG ingestion, acute direct administration of 180 mM MSG induced a significant increase in the number of c-Fos positive cells in the CeA compared with controls and 60 mM MSG-administration (Figure 7E). However, the total number of c-Fos + cells was considerably lower than in rats with prior behavioral tests and PWSI (Figure 6C).

To consider the possibility that PWSI affects c-Fos + cells, the number of positive cells in SHR/Izm rats grown in PWSI was investigated without behavioral tests (Figure 7F). We found that MSG ingestion increased the number of c-Fos + cells in the iNTS (Figure 7G). In addition, a significant increase in the number of c-Fos + cells was observed in the CeA of rats grown in the PWSI

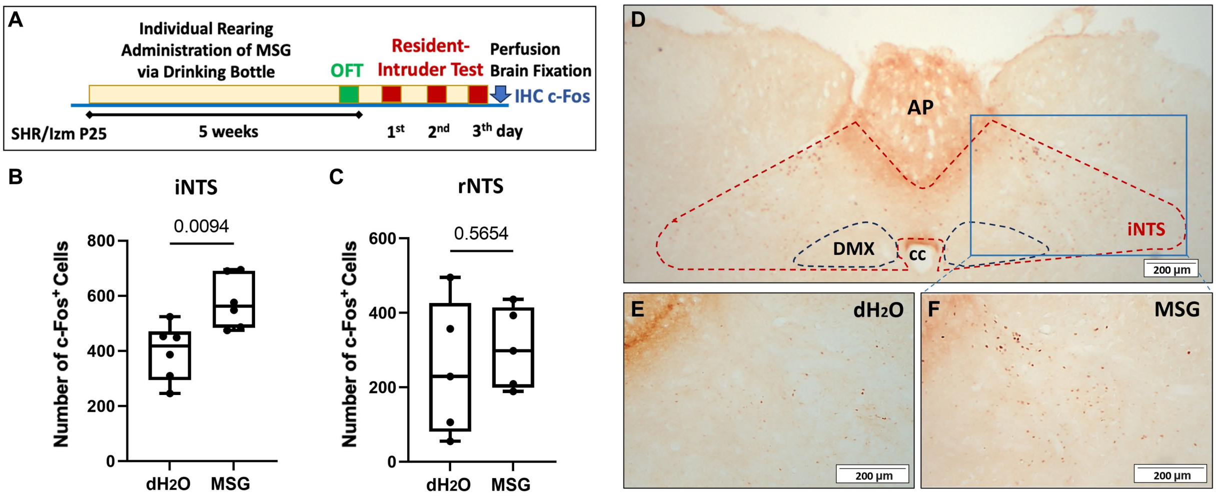

FIGURE 5
The effect of MSG ingestion on the number of c-Fos + cells in the nucleus of the solitary tract. **(A)** Schematic illustration of the experimental timeline. **(B,C)** Significantly increased c-Fos + cells were counted in the iNTS from the MSG group compared with the control group (n = 6/group) **(B)**, while no difference was observed in the rostral part of the NTS (rNTS) between both groups (n = 5/group) **(C)**. **(D–F)** Overview of c-Fos immunoreactivity in the coronal section of the intermediate part of the NTS (iNTS) **(D)** in the control **(E)** and MSG groups **(F)**. Each bar represents the mean ± SEM; statistical analysis was performed using unpaired t-test.

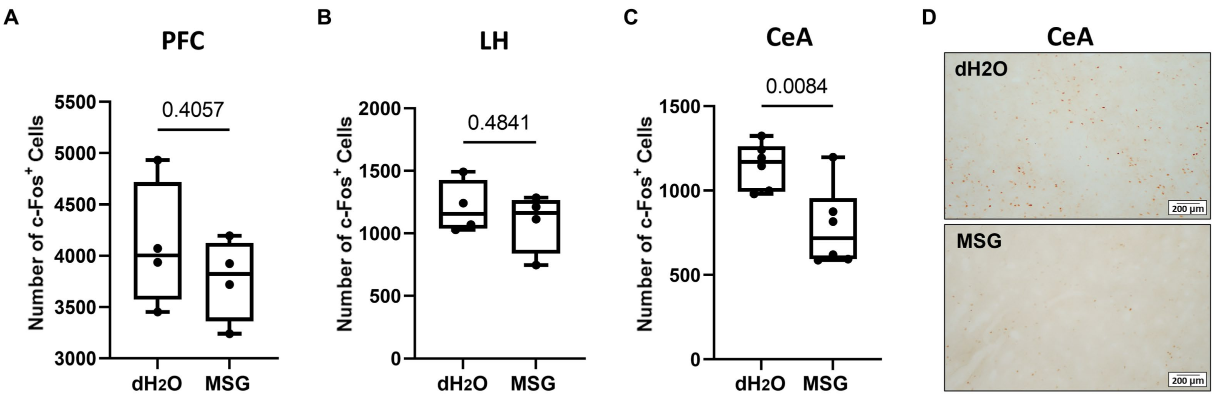

FIGURE 6
The effect of MSG ingestion on the number of c-Fos + cells in the PFC, LH, and CeA. **(A,B)** The number of c-Fos + cells was comparable in the PFC **(A)** and LH **(B)** between the MSG-ingested group (n = 4) and the control group (n = 4). **(C)** The number of c-Fos + cells decreased significantly in the CeA of MSG ingested group (n = 6) compared to that in the control group (n = 6). **(D)** Overview of c-Fos immunoreactivity in the coronal section of the CeA in the control and MSG groups. Each bar represents the mean ± SEM; statistical analysis was performed using Mann–Whitney Test.

(Figure 7H). Interestingly, this enhanced expression in the CeA by PSWI was significantly reduced by ingestion of 60 mM MSG (Figure 7H), indicating that PWSI is a major factor that increases c-Fos + cells in the CeA, whereas MSG reduces the PWSI effect.

3.7 MSG effect on aggression and c-Fos expression in the iNTS and CeA was diminished by vagotomy

Bilateral subdiaphragmatic vagotomy was performed at P23 to verify the importance of gut-brain interactions in the MSG effect (Figure 8A). Vagotomy blocked the effect of MSG on aggression in the resident intruder test; even with MSG ingestion, the duration of attack (Figure 8B), the frequency of aggressive grooming and attack (Figure 8C), and the latency of attack (Figure 8D) were similar to the control level. Vagotomy also diminished the effect of MSG on c-Fos expression in the iNTS (Figure 8E) and CeA (Figure 8F), indicating that the vagus nerve plays an important role in the effect of MSG on aggression and modulation of neuronal activity, not only in the iNTS but also in the CeA.

4 Discussion

In this study, after confirming that SHR/Izm is an appropriate model for aggression and identifying aggression-related brain areas, our hypothesis regarding how gut-brain stimulation by MSG

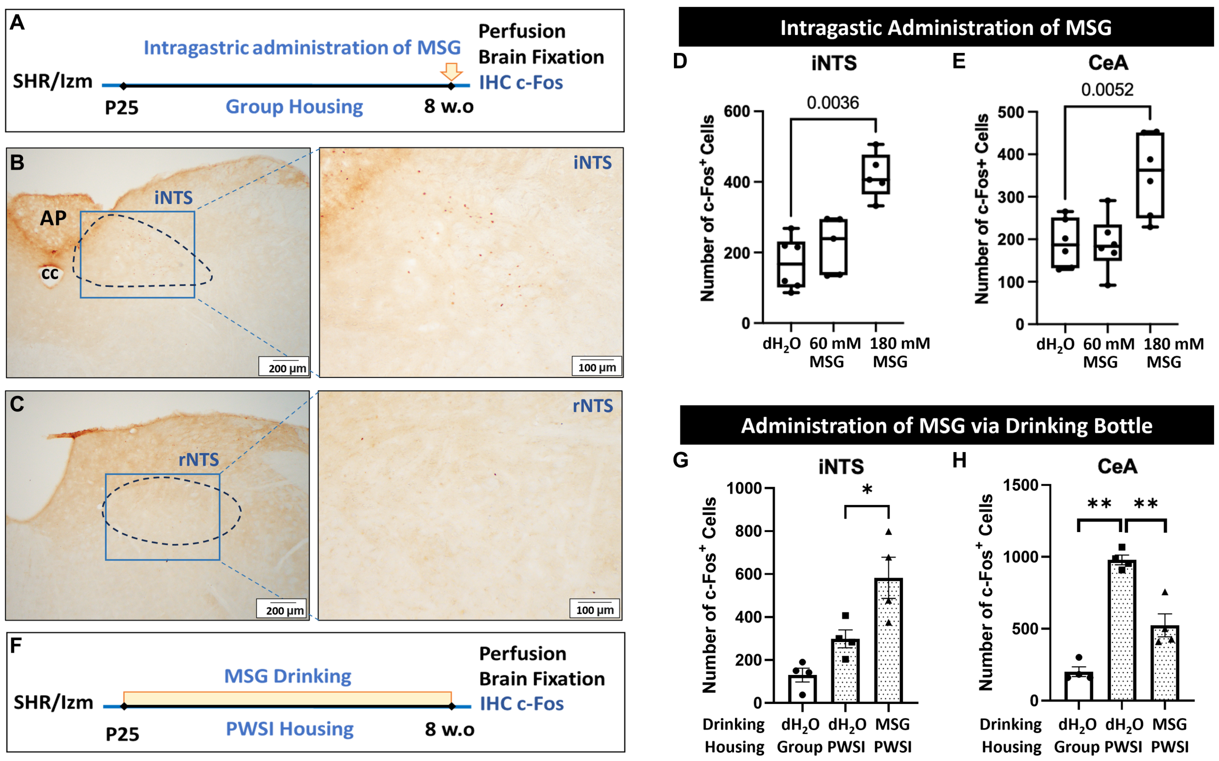

FIGURE 7
Intragastric administration of MSG increased the number of c-Fos + cells in the iNTS and CeA. **(A)** Schematic illustration of the experimental timeline for intragastric administration of MSG. **(B,C)** Overview of c-Fos immunoreactivity in the coronal sections of the iNTS **(B)** and rNTS **(C)**. **(D,E)** Intragastric administration of 180 mM MSG to SHR/Izm rats significantly increased the number of c-Fos + cells in the iNTS **(D)** and CeA **(E)**. **(F)** Schematic illustration of the experimental timeline for MSG administration via a drinking bottle. **(G)** The number of c-Fos + cells in the iNTS increased in the MSG drinking group compared to the control group. **(H)** The number of c-Fos + cells in the CeA was higher in the rats that experienced PWSI than in the rats in group housing, while MSG drinking reduced the number of c-Fos + cells elevated by PWSI. Each bar represents the mean ± SD (*n* = 4–6) and statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons.

potentially influences aggressive behavior was reaffirmed through the resident-intruder test and c-Fos immunostaining.

The level of aggression could vary depending on the rat strain, even though PWSI in SHR/Izm heightens aggression, as reported in Wistar rats and Sprague–Dawley rats (10, 13, 67, 68). SHR/Izm rats are often used as the animal model of ADHD although those lack impulsivity in several paradigms (22, 55, 69). SHR-SP/Ezo rats that were isolated as a SHR substrain, are known to have more impulsivity compared with SHR/Izm rats (70). As aggression is related to impulsivity in ADHD, we assumed that the SHR-SP/Ezo rats are potentially more aggressive. However, our data showed that SHR/Izm rats were more aggressive than the SHR-SP/Ezo rats. Higher motor activity in SHR/SP would be related to less aggression in the resident intruder test, since contact between the resident and intruder is relatively sorter due to the high movement of SHR-SP/Ezo (70). In contrast, Long-Evans rats displayed mild behavior without any attack behavior, despite undergoing the same PWSI conditions.

It is reported that PWSI affect the medial prefrontal cortex (mPFC) by decreasing dendritic density and glial cell number, inducing hyperactivity of glutamatergic and GABAergic neurons, and resulting impairment of excitatory/inhibitory balance (10, 12, 71). In optogenetic studies, the projection from the mPFC to the mediobasal hypothalamus contributes to the quantitative aspects of aggressive biting behavior, whereas the projection from the mPFC to the LH is linked to the qualitative aspects of abnormal aggressive behaviors (72). Therefore, the higher number of c-Fos positive cells in the PFC and LH of SHR/Izm compared to Long-Evans could explain the different aggression levels between these strains. Further studies are needed to understand the underlying mechanisms of PWSI-induced increased neuronal activity in the PFC and LH in aggressive rats, whereas less aggressive rats such as Long-Evans seem insusceptible to PWSI. Factors such as gene interactions and genetic background seem to play a role in the strain and individual differences in aggression (18, 73–75).

Another brain area that appears to play a significant role in aggressive behavior is the CeA, since impulsive aggression occurs when the amygdala (Amy) is overactivated with inadequate regulation from the PFC (47). The CeA, with its complex structure and extensive connections to other areas of the brain, plays a significant role in behavior (47, 49, 54). CeA has previously been reported to be more closely associated with predatory aggression than with territorial or hyperarousal aggression related to PWSI (49). Our data, demonstrating an increased number of c-Fos positive cells in the Amy and PFC of SHR/Izm rats, affirms that this rat strain is a suitable model for aggression in ADHD. The heightened neuronal activity in the Amy and PFC of SHR/Izm rats is consistent with a study that used functional magnetic resonance imaging in children with ADHD. The study found that higher emotional instability ratings correlated with stronger positive intrinsic functional connectivity between the amygdala and rostral anterior cingulate cortex (48).

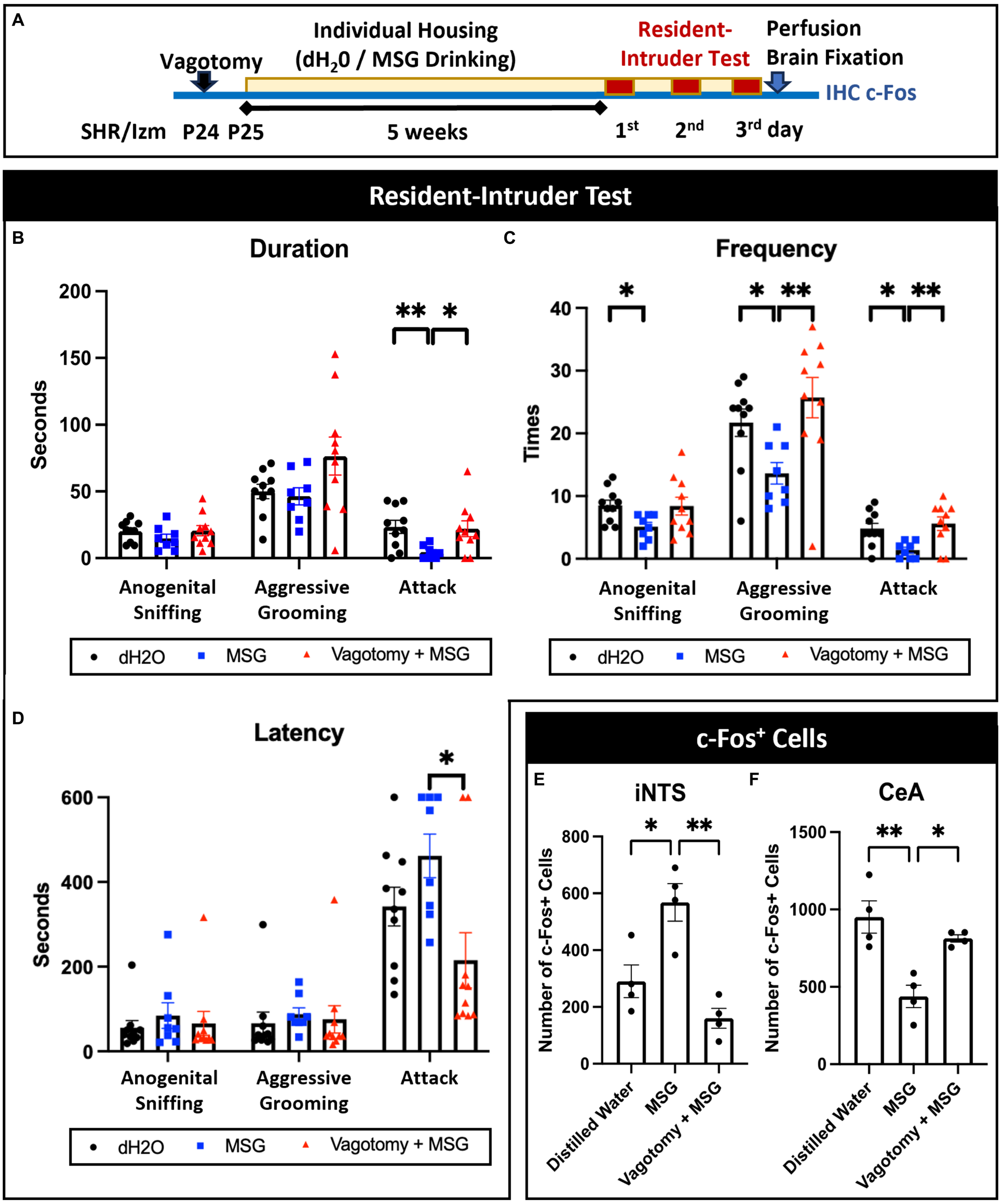

FIGURE 8
Vagotomy diminished the effect of MSG ingestion on aggressive behavior and c-Fos + cells in the iNTS and CeA. **(A)** Schematic illustration of the experimental timeline. **(B–D)** Vagotomy successfully diminished the effect of MSG on the duration of attack behavior **(B)**, frequency of aggressive grooming and attack behavior **(C)**, and latency of attack behavior **(D)**. **(E,F)** MSG ingestion significantly blocked the effect of MSG ingestion on the number of c-Fos + cells in the iNTS **(E)** and CeA **(F)**. Each bar represents the mean ± SEM; *p<0.05, **p<0.01, statistical analysis was performed using Kruskal-Wallis test followed by Dunn's test for resident intruder test (n = 8) and one-way ANOVA followed by Tuckey's multiple comparisons test for c-Fos + cells (n = 4).

Since aggressive behavior is related to Amy hyperactivity, a strategy to reduce this hyperactivity might decrease aggressive behavior. Interestingly, the effect of MSG on reducing attack behavior coincided with a decrease in c-Fos positive number of CeA. However, it should be noted that the reduction of CeA activity after MSG ingestion is detected under PWSI conditions where the CeA is in a hyperactive specific state. Since modulation of the CeA could be one of the potential mechanisms for reducing aggression levels in an ADHD rat model, it would be interesting to know which type of neuron or neurotransmitter in the CeA is affected by MSG ingestion. It was reported that social isolation-induced aggression increases glutamatergic activity by increasing AMPAR expression in the CeA (76, 77). Since the CeA mainly consists of inhibitory GABAergic cells, we hypothesized that MSG ingestion possibly stimulates the activity of inhibitory neurons in the CeA, thereby decreasing its total activity, especially excitatory neurons, under conditions that induce aggression (78). Further studies are required to understand how MSG ingestion modulates neuronal activity in the CeA and ultimately leads to behavioral changes.

It has been previously reported that MSG administration evoked c-Fos activity in the NTS (79, 80). Our results also showed that oral and intragastric administration of MSG increased c-Fos positive cells in the intermediate NTS (iNTS), confirming ascending viscero-sensory stimulation by MSG via the vagus nerve. In contrast, we found no c-Fos-positive cells in the rNTS (terminal of the tongue sensory afferent fiber of the glossopharyngeal nerve) after intragastric MSG administration. As glutamate receptors such as T1R1/T1R3, mGluR4, and mGluR1, are expressed on the epithelial mucosa in the stomach and intestine (81, 82), MSG can activate gut-brain signaling mediated by the vagus nerve that terminates in the iNTS, a part of the caudal NTS (cNTS) at the level of the area postrema (52). The important role of the vagus nerve in the effect of MSG was confirmed by vagotomy, which successfully diminished decreasing aggression and modulation of c-Fos activity in the iNTS and CeA. The gut-brain interaction in nutrient sensory transduction is mediated by two systems: one through an electrically excitable cell known as the enteroendocrine cell, and the other via an indirect system utilizing slow endocrine action of hormones such as CCK. Thus, the brain can perceive gut sensory cues through faster neuronal signaling mediated by the 'Neuropod Cell,' which connects the intestinal lumen to the brain stem in a single synapse (83). As the sugar stimulus from enteroendocrine cells in the intestinal lumen is transduced to vagal neurons using glutamate as a neurotransmitter, it raises an intriguing question: whether the MSG stimulus is also transmitted via Neuropod Cells, and which neurotransmitter facilitates the rapid transfer of sensory signals to vagal neurons upon MSG ingestion (84).

Interestingly, a bidirectional connection between the caudal NTS and the CeA has been reported (51, 54). The presence of CeA projection neurons from the cNTS in bregma levels −14.86 to −13.60, including at the level of AP (iNTS), has been identified in rats, suggesting an ascending efferent system from the cNTS to the CeA (53, 54). Consequently, iNTS activation by MSG via the vagus nerve potentially influences the activity of various brain regions involved in the control of both behavior and physiology (46). Moreover, our data showed that vagotomy successfully inhibited the effect of MSG in decreasing CeA hyperactivity in PWSI. Under normal conditions without inducing aggression (Figures 7A–E), our data showed that MSG increased c-Fos-positive cells in both the iNTS and CeA. Therefore, one possible pathway for the MSG effect based on our data is the projection of neuronal activation from the iNTS inhibiting hyperactivity in the CeA, possibly by the stimulation of inhibitory neurons in the CeA (46). However, we cannot exclude the possibility of indirect mechanisms through other areas of the brain associated with aggression, as the cNTS establishes a broad network involving multiple brain regions, not only the CeA, but also the PBL, PAG, PVH, LC, and BNST (53). A previous study showed that the administration of umami-rich dried bonito broth reduced aggressiveness, correlating with the densities of parvalbumin-immunoreactive neurons in the mPFC, amygdala, and hippocampus. This correlation indicates a potential indirect mechanism that is not mediated by the vagus nerve (85).

In this study, although MSG ingestion did not completely eliminate aggression, the significant decrease in strong aggressive behavior shows the possibility for a promising adjunctive therapy along with other strategies to manage aggression. It has been reported that daily intake of MSG in Europe and United States is 0.3–0.5 g/day while in Asia is 1.2–1.7 g/day (86). In this study, the human equivalent dose (HED) was estimated from the daily intake per kilogram body weight (kgBW/d) of rats, involving allometric scaling by multiplying with the Km ratio of 0.162 and adjusting for the high sensitivity of the taste receptor T1R1/T1R3 in humans by dividing by 20 (82, 87, 88). Based on these data, the MSG daily intake in this experiment is estimated to be equal to 22.55–26.98 mg/kgBW/d in humans (Supplementary Figure S1). This estimation is considered safe according to the population-acceptable daily intake (ADI) of MSG, which is proposed as 32 mg/kg BW/day by the European Food Safety Authority (EFSA), and the "No Observed Adverse Effect Level" dose is 3,200 mg/kg BW/day (89).

5 Conclusion

This study established improved experimental conditions to investigate the detailed brain mechanisms involved in reducing aggression induced by MSG. The resident-intruder test was used to observe aggressive behavior instead of the social-interaction test, and aggression was observed under red dim light during the subjective night. A comparison of isolation-induced aggression was performed in different rat strains, revealing that the ADHD rat model, SHR/Izm, is the most aggressive strain compared to Long-Evans, WKY/Izm, and SHR/Ezo. Immunostaining of c-Fos in aggression-related brain areas indicated that strong c-Fos expression in the mPFC, CeA, and LH may be linked to heightened aggression in SHR/Izm. Interestingly, the effect of MSG on decreasing aggression was confirmed by the resident-intruder test in SHR/Izm, possibly related to iNTS activation via the vagus nerve and modulation of CeA hyperactivity under conditions of PWSI-induced aggression. The effects of MSG on aggression and c-Fos expression in the iNTS and CeA were diminished by vagotomy. The effects of MSG stimulation on iNTS and CeA were also detected by direct single intragastric administration of MSG. The modulation of CeA activity is potentially linked to iNTS activation. Therefore, the iNTS-CeA projection, whether direct or indirect, could be a key mechanism in the effect of MSG on reducing aggressive behavior.

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. Requests to access the datasets should be directed to hhida@med.nagoya-cu.ac.jp.

Ethics statement

The animal study was approved by the committee on animal experimentation of Nagoya City University Medical School. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

DM: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. YN: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. SU: Data curation, Investigation, Methodology, Project administration, Writing – original draft. ST: Methodology, Writing – original draft. TS: Methodology, Validation, Writing – review & editing. NT: Methodology, Validation, Writing – review & editing. C-GJ: Validation, Writing – review & editing. HH: Conceptualization, Funding acquisition, Methodology, Project administration, 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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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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.2024.1356189/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Daily intake of MSG in experimental rats and estimation of the human equivalent dose of MSG. **(A)** The volume of drinking consumed by SHR/Izm in the MSG group (MSG; *n* = 12) and control group (dH_2O; *n* = 12) was measured during five-week PWSI and MSG administration from P25 until P60. **(B)** Daily intake of MSG in the MSG group was calculated from the volume of drinking water containing 60 mM MSG consumed per day. **(C)** Body weight was measured during five-week PWSI and MSG administration period from P25 until P60. **(D)** The dose of MSG administered was calculated based on the daily intake of MSG per rat body weight. **(E)** The human equivalent dose (HED) was calculated from the daily intake per kilogram body weight of rats, involving allometric scaling by multiplying with the Km ratio of 0.162, and the adjusted HED was estimated by dividing 20 regarding the high sensitivity of the taste receptor T1R1/T1R3 in humans.

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Development and validation of Japanese version of alternative food neophobia scale (J-FNS-A): association with willingness to eat alternative protein foods

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Introduction: Food neophobia (FN) is a psychological trait that inhibits one's willingness to eat unfamiliar foods. It is related to the acceptance of insect foods and cultured meat, which are major protein alternatives to conventional meat, and is an important personality trait for understanding the near-future food industry. However, the factor structure of Pliner and Hobden's FN scale (FNS) is unstable due to respondents' cultural backgrounds. Thus, we aimed to develop a Japanese version based on the alternative FNS (FNS-A), the most recent revised version, and to examine its validity.

Methods: Four online surveys (preliminary 1: $n = 202$; preliminary 2: $n = 207$; main: $n = 1{,}079$; follow-up: $n = 500$) were conducted on the FNS-A. For the main survey, Japanese respondents (aged 20–69 years) answered the Japanese version of the FNS-A (J-FNS-A), their willingness to eat (WTE), and their familiarity with hamburgers containing regular protein foods (ground beef, tofu) and alternative protein foods (soy meat, cultured meat, cricket powder, algae powder, and mealworm powder).

Results: Consistent with the FNS-A, confirmatory factor analysis assuming a two-dimensional structure (approach and avoidance) showed satisfactory model fit indices. The mean J-FNS-A score (Cronbach's α for 8 items = 0.83) was 4.15 [standard deviation (SD) = 0.93]. J-FNS-A scores were not associated with age and gender, whereas a greater than moderate association was found with WTE hamburgers containing alternative protein foods ($rs = -0.42$ to -0.33). The strength of these negative associations increased as food familiarity decreased ($r = 0.94$). The test–retest reliability at 1 month was also satisfactory ($r = 0.79$).

Discussion: The validity of the J-FNS-A was confirmed. Higher J-FNS-A scores (mean = 41.51, $SD = 9.25$, converted to Pliner and Hobden's FNS score) of the respondents suggest that Japanese people prefer conservative foods. This scale could predict the negative attitudes toward foods with low familiarity, such as alternative proteins. The J-FNS-A appears to be a useful psychological tool for assessing Japanese food neophobia tendencies and predicting novel food choices of Japanese individuals.

KEYWORDS

food neophobia, alternative protein, entomophagy, clean meat, lab-grown meat

1 Introduction

The global population is projected to reach 8.5 billion in 2030 and 9.7 billion in 2050 (1), and people in low- and middle-income countries are becoming more carnivorous in recent decades. Specifically, global meat consumption *per capita* increased by 25% from 1990 to 2010 and is projected to increase by approximately 14% from 2021 to 2030 (2, 3). Population growth and increasing global meat consumption are driving the demand for meat and threatening food security (3). Furthermore, the development of the livestock industry contributes to global environmental impacts, particularly in terms of carbon and water footprints (4–6), and raises concerns regarding animal welfare (7). Addressing these challenges necessitates a shift in dietary styles to reduce the consumption of conventional meat through vegan, vegetarian, and flexitarian diets (8, 9), as well as the need to shift the use of protein foods from conventional meat to alternative sustainable resources (10, 11) or to more sustainable livestock production systems such as silvopasture, woodland, and rotational grazing (12).

A recent review by Onwezen et al. (13) focused on plant-based soy protein (soy meat) and pulses, insect foods, microalgae proteins, and cultured meat derived from beef muscle cells, as major alternative protein foods, from the three perspectives of novelty, desirability, and plausibility (14) and discussed global consumption trends and preferences (13). Increasing acceptance is crucial for the successful diffusion of novel foods, such as alternative protein foods, that have not traditionally been on the market. In addition to safety, cost, convenience, and the sensory qualities of foods play a pivotal role in their acceptability (13, 15–20). However, sensory preferences for alternative protein foods are expected or recognized to be less favorable than those for conventional meat (beef in many cases), and many alternative protein foods are not well accepted (13, 16, 21–25).

In addition to food-related factors (safety, cost, convenience, and sensory qualities), consumer psychological factors have been identified as potential inhibitors of the acceptance of alternative protein foods (4, 26, 27). Food neophobia (FN) is a major psychological factor related to the acceptance of alternative protein foods. It explains the reluctance to consume unfamiliar foods (28–30). The food neophobia scale (FNS), developed by Pliner and Hobden, allows for the quantification of FN tendencies (31) and has been translated into several languages [e.g., Brazilian Portuguese, Chinese, Finnish, French, German, Korean, Spanish, Swedish; see systematic review by Rabadán and Bernabéu (32)] and is used in research worldwide. Strong FN tendencies are associated with low dietary variety (e.g., fruit and vegetable intake), many disliked foods, low willingness to try new foods, and negative attitudes toward foods from other cultures (33–36). Moreover, there are similar concepts derived from FN, such as motivation to eat new foods (MENF) and food technology neophobia (FTN). MENF represents the willingness to try novel foods in two dimensions (approach and avoidance), which can be measured by the MENF scale (29), which may be a more detailed version of the FNS. In addition, FTN represents fear of novel food technology and can be measured by the FTN scale (FTNS) (37). FTNS scores are more than moderately associated with willingness to try current food technologies (e.g., pasteurization, high-pressure processing, modified atmosphere packaging) and novel food technologies (e.g., triploidy, genetic modification, bioactives). These suppressed behaviors are believed to be based on an organism's survival strategy to protect itself from foods containing allergens and pathogens, and it is believed that strong anxiety and aversion to unfamiliar foods or food technology evoke rejection or avoidance of eating (38, 39).

Many alternative protein foods developed in recent years are unfamiliar to most consumers, as their ingredients and manufacturing processes differ significantly from those of conventional foods. Consequently, FN has been shown to significantly influence eating experiences and/or willingness to eat (WTE) alternative protein foods (40–42). FN has been investigated as an important psychological factor predicting the acceptance of alternative protein foods, which are expected to become more popular, as well as ways to avoid their negative effects and improve eating behavior (43). However, the concept of FN is rooted in Western culture; in East Asian countries, fewer studies have been translated into their own language (e.g., Chinese, Korean, Japanese) (34, 44) and conducted to evaluate the negative impact of FN on the acceptance of alternative protein foods (15, 33) than in Western countries.

To estimate the future diffusion of alternative protein foods in Japan, it is essential to measure Japanese FN. However, studies on Japanese FN are insufficient in terms of both quality and quantity. Even though the English version of the FNS (25) was used with Japanese participants, it lacked language information and translated content and did not assess the internal consistency of the scale or remove certain items (45–49). In the study of Imada and Yoneyama, 14-item Japanese statements related to food neophilia (3 items) and FN (11 items) were selected from 41 statements that were originally generated by 27 Japanese university students, and a factor structure of the Japanese version of the FNS was tested using an exploratory factor analysis (EFA) to verify its validity (50); however, some questions remain, such as 3 items related to the avoidance factor that do not show negative factor loadings for the approach factor. Despite this theoretical paradox, the 14-item score was used as the FN tendency in later studies (51, 52). Alternatively, removing 3 items (two of which were related to the avoidance factor) confirmed a sufficient internal consistency to use as the food neophilia scale (15). This ambiguity in scale content and item differences may lead to difficulties in comparing and integrating the findings on Japanese FN. Even in recent FN research reviews, studies on Japanese populations have not been addressed at all (32, 43).

The origin of these issues may be attributed to differences in culture and time periods. For example, Pliner and Hobden's FNS was developed based on a survey of Canadian university students in the 1990s. Typically, items comprising recently developed psychological scales are aligned with the cultural context of the country in which they were developed, and translation into multiple languages carries the risk of altering their meaning for participants from other cultures (53). Indeed, the FNS has been modified for use in different countries and cultures by replacing words (33, 54, 55) and/or removing certain items (56–59). FN tendencies have weakened worldwide over the past two decades. This is attributed to the impact of globalization, which has increased exposure to food cultures in other countries through international travel, international trade, and restaurants serving foreign cuisine (32). In light of these cultural differences and changing times, certain items in the original FNS parameters, such as "Ethnic food looks too weird to eat" and "I like to try new ethnic restaurants" may no longer be appropriate today. To address these issues, De Kock et al. developed an alternative FNS (FNS-A) by revising and reorganizing the items of the original FNS and excluding items related to respondents' cultural backgrounds (60). Even though the FNS-A included data from students from non-native English-speaking areas (South Africa, Botswana, and Lesotho), the FNS-A has the following key advantages: it confirms factor structure validity through factor analyses (exploratory and confirmatory); it demonstrates reliability (internal consistency and test–retest reliability); it demonstrates construct validity by testing its association with other psychological scales related to the FNS concepts (modified version of the FNS, MENF scale, and FTNS); and it can confirm its predictive validity by testing its association with liking or willingness to try unfamiliar or novel foods.

In light of the above, there is no sufficiently validated psychological scale for quantifying FN tendency in Japanese individuals. Nevertheless, it is expected that a Japanese version of the FNS-A (J-FNS-A) with sufficiently high validities (e.g., factor structural validity, internal consistency, test–retest reliability, construct validity, and predictive validity) will be developed by following the FNS-A, the most recent revised version. The development of a validated J-FNS-A would provide a quantitative assessment of the Japanese FN tendency and allow for a comparison with the results of FN studies around the world. Therefore, the purpose of this study was to develop a J-FNS-A and assess its validity among Japanese participants. The predictive validity of the J-FNS-A was assessed by testing its association with WTE novel foods (e.g., hamburgers containing alternative protein foods as patties) (22, 60–64).

2 Materials and methods

Before starting the survey, we obtained permission to translate the FNS-A into Japanese and use it in academic research from the two authors (corresponding and last) of the FNS-A (60). Our study consisted of two preliminary surveys (250 participants each; final number of participants in preliminary 1: $n = 202$; in preliminary 2: $n = 207$) to verify the validity of the translation of the FNS-A into Japanese, a main survey (1,500 participants; final number of participants in the main survey: $n = 1{,}079$) to verify the validity of the factor structure of the J-FNS-A and the predictive validity of WTE novel foods, and a follow-up survey (500 participants, final number of participants in the follow-up survey: $n = 500$) to test the reliability of the retests.

2.1 Participants

All surveys were conducted using an online questionnaire to recruit respondents from a wide range of age groups and a broad geographical area in Japan [excluding Nagano, where the entomophagy culture is flourishing, (47)]. All the respondents were recruited through a web-based survey company (iBridge Corp., Osaka, Japan). For each of the two preliminary surveys (surveys 1 and 2), 250 Japanese individuals aged 20–69 years (50 individuals in each age group: 20–29; 30–39; 40–49; 50–59; 60–69 years; with an equal gender ratio) were recruited. In the main survey, 1,500 Japanese individuals aged 20–69 years (300 in each age group: 20–29; 30–39; 40–49; 50–59; 60–69 years; with an equal gender ratio) were recruited. In addition, a follow-up survey was conducted 1 month later with 500 Japanese individuals aged 20–69 years (100 in each age group: 20–29; 30–39; 40–49; 50–59; 60–69 years; with an equal gender ratio) responding to the main survey. All respondents were identified by their identity documents, and there were no repeat responses in the preliminary and main surveys.

All surveys were conducted according to the principles of the Declaration of Helsinki. Prior to participation, potential participants received a brief description of the survey and were informed of their right to withdraw at any time. Informed consent was obtained from all participants through an online platform. The survey protocols were exempt from review by the Ethics Committee of the Food Research Institute, National Agriculture and Food Research Organization, Japan, for the following reasons: the survey results must be collected in an anonymous format that does not allow the identification of individuals. The content of the questions should be such that they cause (almost) no psychological stress.

2.2 Preliminary surveys (1 and 2)

To guarantee a reliable translation of the original questionnaire, a double-back translation (English → Japanese → English) of the 8 items constituting the FNS-A (60) was performed using an English editing service (Crimson Interactive Pvt. Ltd.). Double-back translation consisted of three steps: translation from English to Japanese, translation from Japanese to English, and verification of each translation. These three steps were each performed by three bilingual Japanese–English speakers independent from the authors. In addition, the two authors of the FNS-A (60) were then requested to check the translations. After obtaining agreement, the first preliminary survey of the J-FNS-A, including two directed question scale (DQS) items (65), was conducted, and the item–remainder (I-R) correlation coefficients were calculated for each of the 4 items and the corresponding factors (approach and avoidance). Results (valid responses: $n = 202$, including 93 men and 109 women) showed that the Pearson's correlation coefficient for 1 item ("New foods mean an adventure for me," 新しい食べ物は、自分にとって冒険だ。in Japanese) was quite small ($r_{approach} = 0.13$), unlike other items ($rs_{approach} > 0.55$, $rs_{avoidance} > 0.58$). Therefore, after discussing the issue with the two authors (60) mentioned above, "New foods mean an adventure for me" was revised to "Eating new food is an exciting event for me" A second preliminary survey was then conducted (valid responses: $n = 207$, 97 men and 110 women), and greater than large I-R correlation coefficients ($rs_{approach} > 0.69$, $rs_{avoidance} > 0.53$) were found for each item. The internal consistency of the 8 items was also sufficiently high (Cronbach's $\alpha = 0.83$).

2.3 Main and follow-up surveys

The main survey consisted of three sections: the J-FNS-A (section 1), familiarity (section 2), and WTE (section 3), each of which contained protein foods common in Japan and major alternative protein foods that are well known or expected to become popular worldwide. All participants answered sections 1, 2, and 3. The order of all items within each section was randomized (60). In section 1, participants were asked to respond to 8 items of the J-FNS-A. Participants rated the degree of agreement for each item on a 7-point Likert scale (strongly disagree = 1; neither disagree nor agree = 4; strongly agree = 7). In section 2, participants were asked to indicate their familiarity with each of the seven types of hamburgers containing different types of protein foods, such as ground beef, tofu, soy, cultured meat, cricket powder, mealworm powder, and algae powder. Participants rated familiarity using a 6-point scale [not at all familiar (do not know what this ingredient is) = 1, very familiar (eat this burger often, make it often) = 6]. In section 3, participants were asked to respond to questions about their WTE hamburgers containing the same protein food types as in section 2. Participants rated their WTE using a 6-point scale [not at all motivated to eat (definitely do not want to eat) = 1, very motivated to eat (definitely want to eat, definitely want to try) = 6]. Detailed explanation of alternative protein foods and definition of hamburger for participants presented in the web-based survey can be found in the Supplementary Table 1. Two DQS items (including "Please do not press the button and move on to the next item" and "Please select the first option from the right") were included in each section, and responses that violated the instructions were considered to satisfice.

The follow-up survey was conducted 1 month after the main survey and consisted of only 8 items of the J-FNS-A with 2 DQS items.

2.4 Analysis

In the main survey, 421 participants (28.1% of the total sample) were excluded from the analysis because of one or more missing responses, two or more inappropriate DQS responses, or uniform responses in more than 90% of the total sample. The remaining 1,079 participants (505 men and 574 women, mean age = 45.79 years, standard deviation [*SD*] = 13.44) were included in the subsequent analyses. The final sample size remained large after exclusion due to the need to obtain data from a diverse population in Japan. Therefore, the sensitivity of the detection power was calculated using G*Power 3.1 (66, 67) for the correlation test (point biserial model) before starting the analysis. The sensitivity power calculation with a sample size of $n = 1{,}079$, $\alpha = 0.05$, and $1\text{-}\beta = 0.95$ estimated a required effect size of $\rho > 0.11$. The main analyses were conducted to test the validity of the factor structure of the J-FNS-A, compare the familiarity and WTE hamburgers among the different protein food types, and test the predictive validity of the J-FNS-A on WTE hamburgers containing alternative protein foods.

After checking the data distribution (floor and ceiling effects) for each item and the correlation coefficients between items in the J-FNS-A (68), a confirmatory factor analysis (CFA) was conducted to assess the factor structure validity using maximum likelihood estimation following the two-factor structural model (approach and avoidance) (60). The goodness-of-fit index (GFI), comparative fit index (CFI), root mean square error of approximation (RMSEA), and standardized residual mean of squares (SRMR) were calculated as model fit indices. Each of the 4 items corresponding to the approach was inverted in the calculation of the 8-item J-FNS-A score and in the assessment of internal consistency. After assessing the internal consistency using Cronbach's α (69), the mean scores were calculated as FN scores. In accordance with the original FNS-A, after each of the four approach factor items was inversely calculated and combined with the avoidance factor items, the 8-item J-FNS-A (mean: 8 items) score was used for the main analysis. Higher scores indicate a stronger food neophobic tendency. The association between the 8-item J-FNS-A scores and age (20–69 years) was assessed using Pearson correlation analysis. In addition, the gender difference in the 8-item J-FNS-A score was assessed by Welch's *t*-test.

After visually checking each food residual distribution using a normal Q–Q plot, familiarity with and WTE hamburgers containing different protein food types were compared among different protein food types (ground beef, tofu, soy meat, cultured meat, cricket powder, algae powder, and mealworm powder) using one-way repeated measure analyses of variance (ANOVA). Huynh–Feldt correction and Shaffer's post-hoc analysis were performed when appropriate. Furthermore, the similarity of WTE between protein foods was assessed using polycholic correlation analysis. In addition, as a food-level analysis, a Pearson correlation analysis was conducted between the WTE hamburgers (mean of each protein food) and familiarity (mean of each protein food). The association between WTE and age was assessed using polyserial correlation analysis. In addition, the gender difference in WTE for each hamburger was assessed by Welch's *t*-test.

The predictive validity of the J-FNS-A was assessed by calculating the associations between J-FNS-A scores and WTE hamburgers containing different protein foods as patties using polyserial correlation analysis. In addition, to understand the strength of the associations (correlation coefficients between the J-FNS-A score and WTE), a food-level Pearson correlation analysis with familiarity (mean of each protein food) was performed.

No respondents met the exclusion criteria among the 500 follow-up respondents (250 men and 250 women, mean age = 44.81 years, *SD* = 13.96). The test–retest reliability of the J-FNS-A was assessed by calculating the Pearson correlation coefficient of the 8-item J-FNS-A score obtained from the main and follow-up surveys. All statistical analyses were performed using HAD17.202 (70). All tests were two-tailed, and the significance level was set at $p < 0.05$. Effect sizes were reported using the *r* family and interpreted as 0.1 for small, 0.3 for medium, and 0.5 for large, following a previous study (71). In accordance with the sensitivity power analysis, the results are discussed primarily for an effect size of $r > 0.11$.

3 Results

3.1 Factor structure validity of the J-FNS-A

No floor/ceiling effects were observed for any of the 8 items. Correlation coefficients between items within each factor were below $r = 0.7$ ($rs_{approach} = 0.49–0.68$, $rs_{avoidance} = 0.40–0.55$). The validity of the two-factor structural model was tested using CFA, and a CFA-based path diagram is shown in Figure 1. The variance explained by the two factors was 65.3%, and the estimated goodness of fit of the model was satisfactory (GFI = 0.96, CFI = 0.95, RMSEA = 0.087, SRMR = 0.047). The association between two factors (approach and avoidance) was strong ($r = -0.53$). Internal consistencies were all satisfactory ($\alpha_{approach} = 0.80$, $\alpha_{avoidance} = 0.83$, $\alpha_{8\text{-}item} = 0.83$).

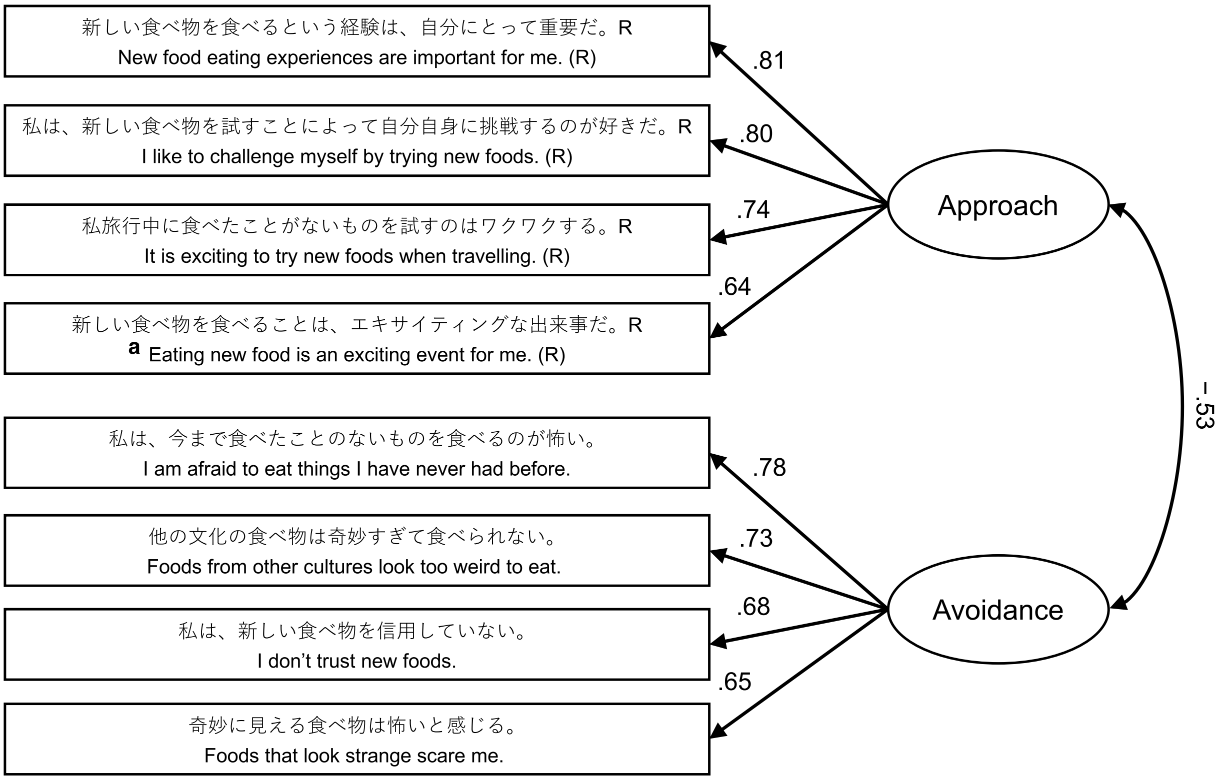

FIGURE 1
Two-factor model of Japanese version of the alternative food neophobia scale based on confirmatory factor analysis. Rectangles, statements of items (observed variables); ellipses, factors (latent variables). The factor loadings for each item and correlation coefficients between factors are presented. The upper 4 items (statements) in the approach factor reflect food neophilic attitudes, whereas the lower 4 items in the avoidance factor reflect food neophobic attitudes. Item "a" was modified from item 4 ("New food means an adventure for me") in the original alternative food neophobia scale. All items and two directed question scale items were presented to the participants in random order.

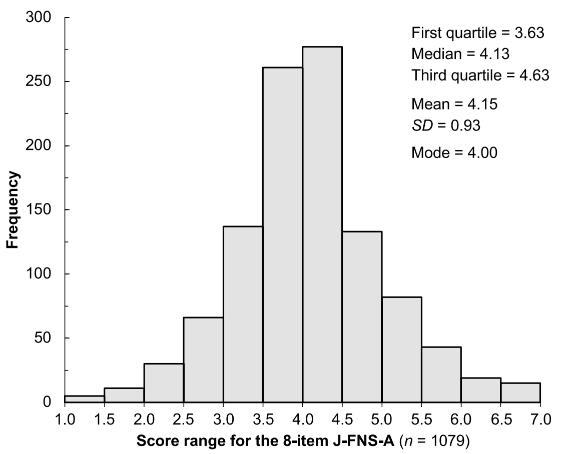

FIGURE 2
Histogram of Japanese version of the alternative food neophobia scale (J-FNS-A) scores obtained from the main survey ($n = 1,079$).

A histogram of the 8-item J-FNS-A scores is shown in Figure 2. The associations between 8-item J-FNS-A scores and age was not significant ($r = 0.007$, $p = 0.83$). In addition, there was no significant difference between men (mean = 4.13, $SD = 0.94$) and women (mean = 4.17, $SD = 0.91$) in the 8-item J-FNS-A score ($t[1051.49] = 0.58$, $p = 0.56$, $r = 0.02$).

3.2 Familiarity with and WTE hamburgers containing various types of protein foods

The results of familiarity with and WTE hamburgers containing different types of protein foods are shown in Figure 3. More detailed results (measures and statistic value) are shown in Supplementary Table 2. The repeated measures ANOVA for familiarity revealed a significant main effect of protein food type. Post-hoc tests for familiarity were all significant. Familiarity was highest for hamburgers containing ground beef and lowest for hamburgers containing mealworm powder. In addition, there was a significant main effect of protein food type on WTE. Post-hoc tests for WTE were all significant. Although there was small difference in familiarity between cricket and algae powders ($r = 0.04$), WTE algae powder was more than moderately higher than cricket powder ($r = 0.40$). Moreover, correlation analysis of WTE revealed significant associations within protein food types (see Supplementary Table 3). Associations were particularly strong ($rs > 0.8$) between tofu and soy meat, cricket powder, and mealworm powder. In addition, food-level analysis revealed a significant association between familiarity and WTE ($r = 0.92$, $p < 0.001$), suggesting that WTE becomes higher as food familiarity increases.

Further correlation analysis revealed a significant association between WTE and age only for cultured meat, indicating a decrease in WTE with older age (see Supplementary Table 4). In addition, several significant differences between men and women were found for WTE (see Supplementary Table 5). WTE tofu and soy meat was

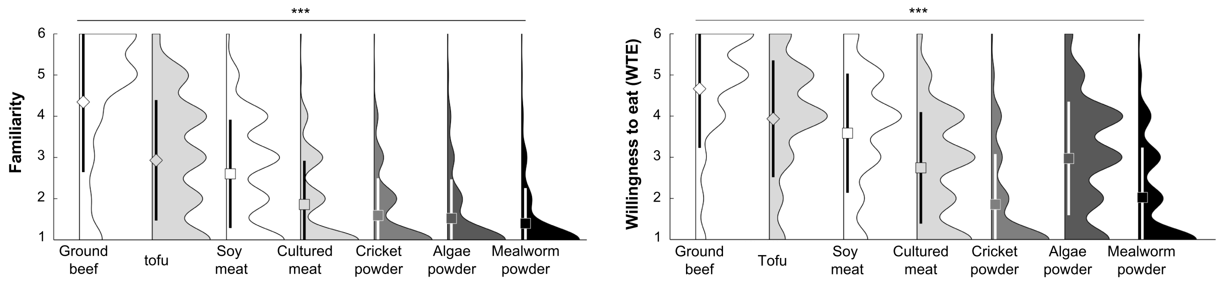

FIGURE 3
Familiarity and willingness to eat (WTE) score for each protein food. Diamond (◇) or square (□) plot, mean; black or white vertical bold line (error bar), standard deviation (*SD*). The main effect of protein food type and *post-hoc* tests were significant. Detailed results are available in Supplementary Table 2.

TABLE 1 Correlation between the 8-item J-FNS-A score and willingness to eat hamburger.

	Patty-protein type	Ground beef	Tofu	Soy meat	Cultured meat	Cricket powder	Algae powder	Mealworm powder
J-FNS-A	Approach	**0.27*****	**0.32*****	0.32***	0.31***	0.30***	0.34***	0.32***
	Avoidance	−0.17***	−0.20***	−0.23***	−0.29***	−0.41***	−0.31***	−0.37***
	8-item	−0.26***	−0.31***	**−0.33*****	**−0.35*****	**−0.42*****	**−0.38*****	**−0.41*****

Correlation coefficients for the polyserial correlation analysis are shown for each calculation method of J-FNS-A scores. The largest coefficients within the various score calculations are in boldface. Statistical significance is indicated by the *p*-value (***$p < 0.001$). J-FNS-A, Japanese version of the alternative food neophobia scale.

higher among women, whereas WTE cultured meat, cricket powder, and mealworm powder were higher among men.

3.3 Predictive validity of the J-FNS-A for WTE alternative protein foods

Correlation analysis was performed to assess the association between J-FNS-A scores and WTE hamburgers with each protein food type (Table 1). Significant associations were found between the J-FNS-A scores (approach, avoidance, and 8-item) and WTE. The J-FNS-A approach score was strongly associated with ground beef and tofu, which have a relatively high familiarity for Japanese people. In contrast, the 8-item J-FNS-A scores were strongly associated with less familiar alternative protein foods (soy meat, cultured meat, cricket powder, algae powder, and mealworm powder).

In addition, as a food-level analysis, a correlation analysis was conducted on the associations between (J-FNS-A score, WTE) and familiarity (Figure 4). The results showed that the strength of these associations weakened with increasing familiarity ($r = 0.94$, $p = 0.002$).

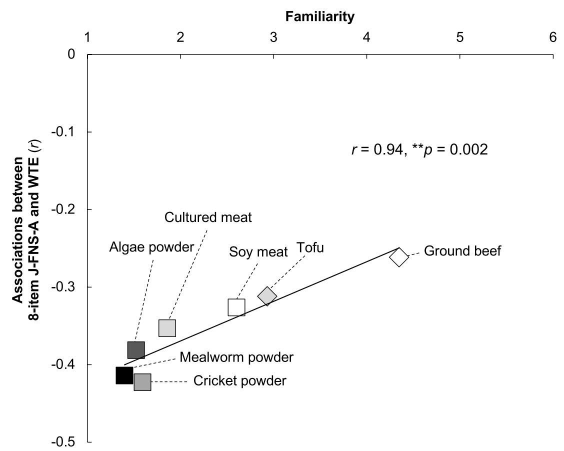

FIGURE 4
The relation between average familiarity score for each protein food and the correlation coefficients (8-item Japanese version of the alternative food neophobia scale score and willingness to eat) are shown. Statistical significance is indicated by *p*-value (**$p < 0.01$).

3.4 Test–retest reliability of the J-FNS-A

The internal consistency of the 8 items was sufficiently high (Cronbach's $\alpha = 0.87$). The mean (*SD*) 8-item J-FNS-A scores for the 500 follow-up respondents were 4.17 (0.93) for the main survey and 4.27 (0.99) for the follow-up survey. There was a significant correlation between the 8-item J-FNS-A scores in the main and follow-up surveys ($r = 0.79$, $p < 0.001$).

4 Discussion

This study examined the validity of the newly developed J-FNS-A by conducting four web-based surveys among Japanese participants. Factor structure validity, internal consistency, test–retest reliability, and predictive validity were satisfactory for the J-FNS-A. The Japanese version of the FNS-A has demonstrated its potential to quantify FN tendencies in Japanese individuals.

4.1 Validities of the J-FNS-A

In preliminary survey 1, 8 items from the original FNS-A were translated into Japanese and tested among Japanese individuals, but 1 item expected to contribute to the approach factor ("New foods mean an adventure for me") was weakly related to the other 3 items ($r = 0.13$) and required minor wording modifications. This may be explained by the fact that the word "adventure" ("冒険" in Japanese) is not necessarily associated with positive feelings for Japanese respondents. This kind of change in meaning with translation is considered an unavoidable issue when conducting surveys with different national (cultural) groups (53). This may also be related to the fact that the FNS-A was developed primarily using data from relatively young and well-educated South African students (54). Nevertheless, the 8 items of the J-FNS-A in this study were developed using data from a broad age range (20–69 years, 46.8% men and 53.2% women) and a wide range of geographic areas, except Nagano (47), and are sufficiently acceptable for most of the Japanese population.

Furthermore, EFA and CFA are considered important in assessing the validity of a measurement when modifying an existing psychological scale (32). Therefore, CFA was performed on the J-FNS-A, which showed sufficient model fit indices with a two-factor structural model similar to the FNS-A. Contrary to the original FNS (31), which shows a one-factor structural model, numerous FN studies have confirmed a two-factor structure because of differences in the translation and/or cultural backgrounds of respondents (34, 58, 72–75). However, instead of following the CFA results, a previous study (60) noted that the original FNS (31) has proven useful in a massive number of research studies as a single continuous scale and recommend inverting the approach factor score and converting it to an eight-item mean or 10 times the mean (which allows comparison with the original FNS). Indeed, our correlation between the two approach and avoidance factors was large ($r = 0.53$), as in the FNS-A (60), and for internal consistency, the eight items were large enough ($\alpha = 0.83$). This coincides with the average Cronbach's alpha coefficient for the four surveys reported previously (60). Furthermore, the internal consistency (Cronbach's alpha coefficient) of the three surveys (preliminary 2: $\alpha = 0.83$, main: $\alpha = 0.83$, follow-up: $\alpha = 0.87$) was generally higher than the previously reported Japanese translation of Pliner and Hobden's FNS (45, 47–49), despite including a smaller number of items. The correlations between the 8 items of the J-FNS-A were not very strong ($rs = 0.09$–0.68) considering a single continuous scale, suggesting that each of the 8 items successfully captures various characteristics of FN in Japanese. Accordingly, the main analysis in this study was conducted using the mean of the 8 items (8-item J-FNS-A score). The 1-month test–retest validity of the J-FNS-A score ($r = 0.79$) was almost identical to the 2-week test–retest reliability of the FNS-A ($r = 0.82$) (60).

The mean (*SD*) J-FNS-A score of the 1,079 Japanese participants was 4.15 (0.93). This is significantly higher than the mean scores (ranging from 2.6–2.9) of the three surveys conducted among university students using FNS-A in South African countries (60). In addition, according to De Kock et al. 2022 (60), FNS-A scores can be converted to Pliner and Hobden's original FN score. The J-FNS-A score (mean = 41.51), multiplied by 10, was notably higher than previous FN scores worldwide (32). Furthermore, this score is higher than the score [$n = 2{,}935$, mean = 29.99; calculated weighted average by the authors from five studies (33, 34, 44, 76, 77)] obtained by recent studies in East Asian countries (China and Korea). This indicates that only 8.53% of Japanese (92 of 1,079 respondents) are below the East Asian (Chinese and Korean) average. Our result seems to reflect the conservative attitude of Japanese people toward eating novel foods and shows that the FN tendency prevails more in the Japanese population than in other East Asians. However, this tendency may merely reflect the characteristics of the Japanese response style toward the magnitude scale (scale anchors) (78–80) and will not be discussed further in this study. Instead, this study confirmed the association of the J-FNS-A with external factors such as age, gender, and WTE novel foods. According to the results, the 8-item J-FNS-A scores were not related to age ($r = 0.007$) or gender ($r = 0.02$). A consistent relationship between the FN and age has not been reported in previous studies (32). The FN model of defense against unfamiliar/novel foods containing allergens and pathogens suggests that as one ages and acquires knowledge and experience, FN decreases. In fact, it has been reported FN is maximized during childhood and slowly declines during adolescence (30, 72, 81). However, it has been reported that FN tends to increase or remain constant with age (73, 82, 83). This is due to the fact that new, unfamiliar foods are observed daily as a result of globalization in recent years (84), but no specific trend was observed in Japanese participants in the present study. The relationship between gender and FN in Japanese individuals was similar to that previously reported for age. Some reports suggest that women have a weaker FN tendency because they are more involved in purchasing and preparing food (85, 86); however, in general, no gender effects have been observed (32, 87, 88). Given the wide age range and large sample size, our results showed that the FN tendency had almost no association with age and gender in Japanese individuals.

The strength of the association between J-FNS-A scores and WTE hamburgers containing alternative protein foods was above moderate ($r = -0.42$ to -0.33) in this study. This replicates the findings that hamburgers with plant-based patties were tested as unfamiliar foods (60). It also replicates studies using the original FNS (73, 89–91). The strength of the association between the J-FNS-A score and the WTE hamburgers made with alternative protein foods reflects the magnitude of the negative effect of FN on food acceptance. Therefore, by conducting a food-level analysis, the results robustly showed that the effect of FN increases from ground beef hamburgers, with relatively high familiarity, to mealworm hamburgers, whose ingredients are not well known (Figure 4). This finding reflects the concept of FN (28, 31) and clearly explains how exposure to food, rather than its sensory characteristics, is essential for its acceptability (92–94). Overall, we demonstrated that the J-FNS-A predicts individual- and food-level WTE unfamiliar/novel foods, which is consistent with previous studies. In addition, the predictive ability of the J-FNS-A was improved by combining the approach and avoidance factors into a continuous FN tendency rather than separating them (Table 1). This supports further use of the 8-item J-FNS-A calculation method. This study focused on alternative proteins as novel foods for the predictive validation of the J-FNS-A. It has been shown that the strength of the FN tendency is related to the WTE functional and healthy foods (95–97) and the amount of fruits and vegetables consumed (98–102), suggesting that picky eating is associated with FN. The J-FNS-A shows potential not only for future food development but also for helping people make healthier dietary decisions.

This study will make many contributions to future Japanese FN studies, but it also has limitations. The key limitation is that this study did not directly compare the Japanese translation of Pliner and Hobden's FNS with the J-FNS-A. De Kock et al. proposed the strength of the FNS-A by directly comparing the FNS-A and the modified FNS (almost same as the FNS) with the same respondents to determine the difference in internal consistency and the conversion calculation method for FNS scores. However, this study could not compare the Japanese translation of the FNS with the J-FNS-A because no Japanese translation of the FNS has been reported in previous studies. The J-FNS-A itself has been validated in numerous tests through four surveys and is a sufficiently useful psychological scale. However, for a detailed comparison with the results of previous FN studies conducted worldwide, a direct comparison with the FNS score should be made in the future. This would allow for a comparison of model fit based on CFA, which is more than just an indirect comparison of internal consistency. It is expected that this point will be addressed in future studies.

4.2 WTE alternative protein foods

In the process of developing the J-FNS-A, Japanese attitudes toward a variety of alternative protein foods were collected. In this section, we focus on the effects of protein food types on WTE rather than on psychological factors (i.e., FN). Recently, comprehensive research on various alternative protein foods was conducted among 5,000 Japanese individuals (25). Although similar in content, Takeda et al. (25) used alternative protein foods themselves as an example, whereas the difference in this study ($n = 1,079$) was the use of hamburger patties as the main ingredient. Hamburgers are a food item often used in sensory evaluation or alternative protein food studies (22, 60–64). In other words, alternative protein foods are expected to become commonly eaten in the near future. Hence, we primarily discuss the similarities and differences with the study of Takeda et al. (25).

The comparison results among alternative protein foods showed that the WTE hamburger was highest for soy meat, algae powder, cultured meat, and insect powder (crickets and mealworms), in that order, which is consistent with the results of Takeda et al. (25) and those for other countries (13, 21, 23). Our results indicate that the WTE protein substitutes is consistent even when the food recipes are different. However, the WTE hamburger containing soy meat was lower than that for hamburgers containing traditional ground beef or tofu. Tofu is a traditional dietary protein food made from soybeans (103) and is expected to become popular worldwide as an alternative protein food (104). In Japan, it is not traditional to eat tofu as a hamburger patty, but as revealed by food-level analysis, familiarity with the food itself, rather than the food recipes, may have contributed to the greater WTE. In addition, the similarity of food ingredients may also be related to WTE specific alternative protein foods. In this study, strong associations ($r = 0.82$) were found between tofu and soy meat, cricket powder, and mealworm powder for WTE, suggesting that each of these pairs is perceived similarly by Japanese consumers. These similarities among protein foods are conceived to exist since the impressions of alternative proteins are not uniform but are captured as multiple groups (clusters). These similarities are consistent with the study evaluating alternative proteins in terms of the evaluation, potency, and activity dimensions (105), which revealed that the Japanese categorized alternative proteins into plant-based and animal-based sources (106). Moreover, the process of making soybeans into a meat-like food is probably more familiar to the Japanese than to residents of Western countries. There is a type of vegetarian cuisine in Japan called *shojin ryori*, in which soybeans are often used and cooked to resemble foods made with real meat (15). It was originally invented in the 13th century for Japanese Buddhist monks who were forbidden from eating animal-based foods (meat, fish) for religious reasons, and it still draws attention today as a healthy food (107). Furthermore, consistent with Takeda et al. (25), the Japanese appear to have a higher WTE hamburgers containing algae than people from other countries. Specifically, despite a small difference in the familiarity of hamburgers containing cricket powder and algae powder ($r = 0.04$), the WTE hamburger containing algae powder was more than moderately higher than that for the hamburger containing cricket powder ($r = 0.40$). This result deviated from the regression line ($r = 0.92$) between familiarity and WTE in the food-level analysis. According to Takeda et al. (25), this difference in Japanese attitudes toward algae and insects is because respondents associate algae with seaweeds (e.g., nori, wakame, sea lettuce), which they eat in their daily lives as a similar food, even though they are not familiar with the algae themselves. Hence, it is reasonable to assume that even though entomophagy is a traditional practice in certain regions of Japan (47), it is easier for most Japanese individuals today to associate it with strange-looking creatures that exist around them than with food, and thus, the Japanese are not motivated to eat them. The above positive attitudes of the Japanese toward tofu, soy meat, and microalgae were interpreted as evidence that the automatic categorization of foods based on cultural background, such as familiarity with ingredients or making processes (recipes), influences their WTE and/or acceptance.

Furthermore, regarding the gender difference, tofu and soy meat were preferred by women ($r_{tofu} = 0.15$, $r_{soymeat} = 0.07$), whereas men preferred cultured meat ($r = 0.10$) and insect powder ($r_{cricket} = 0.14$, $r_{mealworm} = 0.08$), confirming that plant-based proteins are preferred more by women, similar to the findings of Takeda et al. and other studies conducted in other countries (22, 108). Indeed, from a consumer segmentation perspective, it is important to clarify the relationships between demographic characteristics, such as gender and age, and acceptance (14, 16, 61, 109). However, the difference in gender effects is weaker than that in consumers' FN effects ($rs < -0.33$). In addition, this study found similar results for WTE for hamburger patty ingredients and ingredients alone. However, this still raises a question about the methodology of this research. This study provided only a brief written description of the alternative protein and hamburger recipe to the participants and did not use any pictures or other information (price, safety, health benefits). This methodological concern implies that this study examined the effect of the quality (impression) of the alternative protein on WTE, rather than the quantity of the alternative protein (percentage of alternative meat replaced by conventional meat). In a recent study examining the consumption of insect-, plant-, and conventional meat-based hamburgers, food information (main composition, eating experience) was found to influence liking, perceived quality, and perceived nutritiousness of the alternative protein food (110). In addition, visual food examples [using food images, serving real food (111)] have the potential to increase the predictive validity of the J-FNS-A by eliciting evaluations (WTE) based on past food experiences. Based on these findings, future studies (surveys and sensory evaluations) investigating the relationship between the J-FNS-A and alternative protein foods will need to carefully consider the methodology of food presentation according to the research question. Given the pre-tasting information (priming) effect, it is expected that there are more ways than food design to motivate consumption of alternative proteins, even among consumers with strong FN.

5 Conclusion

In this study, the J-FNS-A was developed and validated through four surveys conducted among Japanese respondents to assess construct validity, internal consistency, test–retest reliability, and predictive validity. The results showed that the J-FNS-A successfully predicted the WTE of unfamiliar foods, using alternative proteins such as insect foods and cultured meat as examples. This study represents the first successful development and validation of an FNS adapted to the Japanese population. The J-FNS-A allows for the quantitative assessment of FN tendencies among Japanese consumers, providing a useful psychological tool for understanding cultural differences in food attitudes and increasing the acceptance of alternative protein foods as well as new foods to be developed soon. Going forward, it is essential to encourage collaboration between researchers developing new foods using novel technology and those exploring eating behavior from a cultural and human sciences perspective. Such collaboration will deepen understanding of consumer food preferences and promote acceptance of novel foods in the Japanese market.

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 ethics committee of the Food Research Institute, National Agriculture and Food Research Organization, Japan. 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

MK: Data curation, Methodology, Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Formal analysis, Conceptualization. MN: Writing – review & editing, Software, Formal analysis, Data curation. FH: Writing – review & editing, Validation, Supervision, Investigation. YK: Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Conceptualization.

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Conflict of interest

The authors declare that this study was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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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.2024.1356210/full#supplementary-material

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Contribution of kelp dashi liquid to sustainable maintenance of taste sensation and promotion of healthy eating in older adults throughout the umami-taste salivary reflex

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Introduction: Taste decline, including taste loss in older adults, leads to malnutrition and frailty. In a super-aging society, improving taste decline and maintaining taste sensation are crucial for the wellbeing of older adults. Hyposalivation frequently affects older individuals and is the leading cause of taste decline in older adults. Treating taste decline, including taste loss, in older adults presents challenges due to the limited sustainable methods for increasing saliva production, except for drug therapy, which may lead to adverse effects. Umami-taste stimulation results in a prolonged increase in both the whole salivary flow rate (WF), more than 90% of which is secreted from the major salivary glands, and the minor salivary gland flow rate (MF) in healthy volunteers through the umami-taste salivary reflex. We hypothesized that umami-rich kelp dashi liquid (KDL), commonly used in Japanese cuisine, may alleviate taste decline and sustain normal taste sensation in older adults with hyposalivation. This study investigated whether KDL stimulation could improve taste decline.

Materials and methods: A non-randomized controlled trial was conducted at the dental department of a university hospital, involving those who presented with dry mouth between May 2017 and December 2021. Before and after repeated KDL stimulation, characteristics like changes in WF and MF, the recognition thresholds (RTs) for five basic tastes, and subjective eating and swallowing difficulties were assessed. Statistical comparisons were performed between the values measured before and after KDL stimulation.

Result: A total of 35 older patients were included. Patients with reduced MF and with or without reduced WF exhibited umami-taste loss. Repeated stimulation with KDL increased MF and WF and improved taste loss, including umami, decreased RTs, and normalized each taste. Furthermore, subjective taste impairment, subjective eating and swallowing difficulties, and burning sensations in the oral mucosa were alleviated.

Conclusion: These findings indicate that KDL stimulation improved umami-taste loss and normalized each taste sensation, further alleviating eating difficulties via the umami-taste salivary reflex. Importantly, umami-taste loss was also observed in patients with normal WF but decreased MF, who are typically not diagnosed with hyposalivation. Therefore, KDL has the potential to sustain taste sensations and promote healthy eating habits in older individuals.

KEYWORDS

kelp dashi liquid, umami-taste loss, recognition thresholds of five basic tastes, older adults, umami-taste salivary reflex, subjective eating and swallowing difficulties, minor salivary gland flow rate

1 Introduction

Taste decline, including taste loss in older adults, can result in inadequate nutritional intake (1) and diminished appetite, ultimately leading to frailty (2, 3). Frailty represents a transitional state between a healthy and dependent state, and with suitable interventions, it can be reversed to restore health. Therefore, addressing taste loss through treatment to restore normal taste sensation could mitigate malnutrition, help older adults regain wellbeing, and reverse frailty. In a super-aging society, addressing taste loss and preserving normal taste sensations have emerged as critical concerns for the wellbeing of older individuals (4). In contrast, hyposalivation is a prevalent symptom among older adults and remains a leading cause of taste loss in this demographic (5). It has been reported that the whole salivary flow rate (WF) is decreased in older individuals with hypogeusia (6). This condition often results from causes such as the side effects of prescription drugs and age-related degeneration of the salivary glands, among others (7–9). Hyposalivation is also associated with malnutrition and frailty (6, 10, 11). Taste decline and taste loss are strongly related to hyposalivation for the following reasons: (1) impaired arrival of tastants to the taste pores and microvilli of taste buds due to decreased saliva, and (2) impairment of taste receptors due to a decrease in organic components contained in saliva (mucous glycoprotein, secretory immunoglobulin A [IgA], growth factor, etc.) (10). Consequently, frailty reported to be associated with hyposalivation may be related to taste decline and taste loss. Increasing saliva levels may help improve taste decline and loss and maintain normal taste sensation in older adults, given that hyposalivation is a common symptom in this demographic.

Saliva is secreted from the major and minor salivary glands. Reduced WF has been the primary focus when treating dry mouth, as the majority of whole saliva, approximately 90% or more of whole saliva, originates from the major salivary glands. However, in recent times, there has been a growing recognition of the presence of dry mouth with normal WF (12–14), which is traditionally not diagnosed as hyposalivation. This has shifted attention toward the MF. Although the minor salivary glands contribute to approximately 8% of whole saliva (15), they are widely distributed throughout the oral mucosa (16). Saliva from these minor salivary glands is rich in mucin (17, 18) and secretory IgA (19). Importantly, it is continuously produced both when stimulated and during rest periods (15).

However, to date, the relationship between taste decline, including taste loss and MF has not been elucidated.

Treatment of taste decline, except for drug therapy, in older individuals is difficult due to the lack of a sustainable increase in saliva production; however, drug therapy may cause side effects. Current drug therapies for dry mouth primarily stimulate the parasympathetic nerve to enhance saliva secretion but can lead to side effects such as sweating, nausea, headaches, and vomiting (20). Older adults are more likely to experience these side effects. As a solution to this problem, treatments involving natural products such as plant extracts, including ginger (21, 22), and the treatment method involving the use of the gustatory-saliva reflex to increase the amount of saliva secretion (23–25) have been explored. Among the five basic taste stimuli, umami stimulation elevates both WF and MF to a greater extent than sweet, salty, or bitter stimuli and to a comparable extent to sour stimuli. In particular, the effects of the umami taste persist longer than those of the other four basic tastes in healthy volunteers (24, 25). Umami is effective for increasing WF and MF. Based on these results, we considered that a therapy for taste decline, including taste loss, should be developed using umami. Kelp dashi liquid (KDL), a staple of Japanese cuisine, is rich in umami due to its high glutamate acid content while also containing minimal amounts of other nutrients (26). We hypothesized that the umami-rich KDL could aid in improving taste decline, including taste loss and sustaining normal taste sensation in older adults with hyposalivation, which is a prevalent condition in this demographic.

This study aimed to investigate whether repeated stimulation with KDL could improve taste sensation by increasing MF and/or WF in older patients with dry mouth. Additionally, the research aimed to determine whether KDL stimulation could serve as a sustainable method to maintain healthy taste sensations.

2 Materials and methods

2.1 Study design and patients

To identify strategies for addressing taste decline, including taste loss in older adults, we studied the intricate relationships between hyposalivation (decreased saliva), the most common cause of taste disorders in this demographic, and taste decline. Moreover, we investigated the effectiveness of KDL stimulation as a potential method for improving taste decline. A non-randomized controlled trial was conducted, which included 54 patients with dry mouth who visited the Dentistry Division of Tohoku University Hospital with complaints of dry mouth sensation between May 2017 and December 2021. The study included patients who were

≥20 years old and had decreased minor salivary secretion. The exclusion criteria were: (i) inability to answer questions and provide written consent due to cognitive decline; (ii) oral cancer, or history of radiotherapy in the head and neck, or chemotherapy; (iii) severe ulcerate, lichenoid, or herpetiform oral mucosal disease; (iv) allergies or hypersensitivity to kelp; and (v) patients with iodine contraindication diseases, such as thyroid disease, pulmonary tuberculosis, and Juhring's dermatitis herpetiformis.

To clarify the relationship between minor salivary secretion and the efficacy of KDL in improving oral dryness, we divided patients into the following two groups: (i) the normal whole saliva secretion flow rate with low minor saliva secretion flow rate (NWS-LMS) group, in which patients only had dry mouth sensation, and (ii) the low whole saliva secretion flow rate with low minor saliva secretion flow rate (LWS-LMS) group, in which patients had hyposalivation.

The recommended sample size was calculated based on preliminary experiments using the statistical software JMP R-Pro 17. According to the results of preliminary experiments in patients, the number of patients with increased MF caused by repeated stimulation with KDL was 3 out of 5 patients in the NWS-LMS group and 4 out of 7 patients in the LWS-LMS group. Therefore, by setting the significance level to 0.05, the power to 0.8, and the hypothetical difference value to 0.2, the sample size comprised eight patients in the NWS-LMS group and 13 patients in the LWS-LMS group.

This study was approved by the Ethics Committee of the Tohoku University Graduate School of Dentistry (Ethics No. 2016-3-027) and was conducted following the Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects.

2.2 Procedure

The experimental procedure was performed as shown in Figure 1. Patients determined to have decreased MF via measurements were explained the details of the study, and they provided informed consent. On another day after registration, we measured the MF, WF, and recognition thresholds (RTs) for the five basic tastes—sweet, salty, sour, bitter, and umami—and assessed the intensity/severity of various dry mouth-related symptoms. All patients were given similar instructions, i.e., to keep 25 mL (divided into 2 or 3 times) of water or KDL (10 g/500 mL, or 40 g/500 mL) in the mouth and gargle for 30 s, 10 times daily (example: 3, 4, and 3 times in the morning, afternoon, and evening, respectively) for 2 weeks, savoring the taste thoroughly. Subsequently, patients were given a form to record the number of times they gargled each day and were instructed to bring this record form, along with the KDL that they had made and used, the next day after completing the 2-week gargling stimulation. On the day after each repeated gargle stimulation with water or KDL gargling, we measured the MF, WF, and RT for each of the five basic tastes and assessed the intensity/severity of various dry mouth-related symptoms in the same way as on the day before the research started (Figure 1). The repeated gargling stimulation was performed for 2 weeks, first with water, followed by 10 g/500 mL KDL, and finally with 40 g/500 mL KDL. The patients were instructed to prepare the KDL as follows: finely chop the kelp that you have at home (10 g or 40 g), put it in a plastic bottle with 500 mL of water, and leave it at room temperature overnight. After preparation, it was stored in the refrigerator, and when used, it was returned to room temperature and used for gargling. KDL contains amino acids, such as glutamic acid, which are nutrients that can promote the growth of microorganisms; therefore, KDL spoils easily. To ensure that the participants used KDL safely, pre-prepared KDL was not provided to them; instead, the participants made the KDL at home using a common recipe provided to them. The participants prepared fresh KDL regularly every 2 days at home using this recipe.

2.3 Measurements of MF and WF

The MF was measured using an electronic device that measures the impedance of a fine filter paper (27). The patient's cheeks and lips were held with a cheek retractor; the accumulated saliva was absorbed with a roll wattle after air-drying the lower lip; a 10×10 mm filter paper (Toyo Roshi Kaisha, Ltd., Tokyo, Japan) was placed on the center of the lip, 5 mm from the bottom of the oral vestibule; and the saliva secreted from minor salivary glands was absorbed for 1 min. The filter paper was fixed to the central part of the electrode, and the displayed electrical potential difference value was read. The MF was obtained using the calibration curve and conversion formula from the potential difference value of the filter paper that absorbed the saliva. A decreased MF was defined as a cut-off value of < 1.0 μL/cm^2/min, based on our previous research (28).

The WF was measured using the gum test (29), in which the patients chewed gum and spit out the saliva secreted into a plastic tube for 10 min. A decreased WF was defined as a flow rate of $<$ 10 mL/10 min (29).

The MF and WF were measured in all patients 1 h after meals and tooth brushing.

2.4 Measurements of the RTs of the five basic tastes

The RTs of the four basic tastes—sweet, salty, sour, and bitter—were measured using Taste Disks® (Sanwa Chemical Co., Ltd., Nagoya, Japan) that consisted of five concentrations of the test solutions for each taste quality (30–32). The concentrations of the four basic tastes were as follows:

i) Sweet (sucrose): 8.7 mM (No. 1:S1), 73.0 mM (No. 2: S2), 292.1 mM
(No. 3: S3), 584.2 mM (No. 4: S4), and 2,337.1 mM (No. 5: S5).
ii) Salty (NaCl): 51.3 mM (No. 1:N1), 213.8 mM (No. 2: N2), 855.5 mM
(No. 3: N3), 1,711.1 mM (No. 4: N4), and 3,422.3 mM (No. 5: N5).
iii) Sour (tartaric acid): 1.3 mM (No. 1:T1), 13.3 mM (No. 2: T2), 133.2 mM
(No. 3: T3), 266.5 mM (No. 4: T4), and 533.0 mM (No. 5: T5).

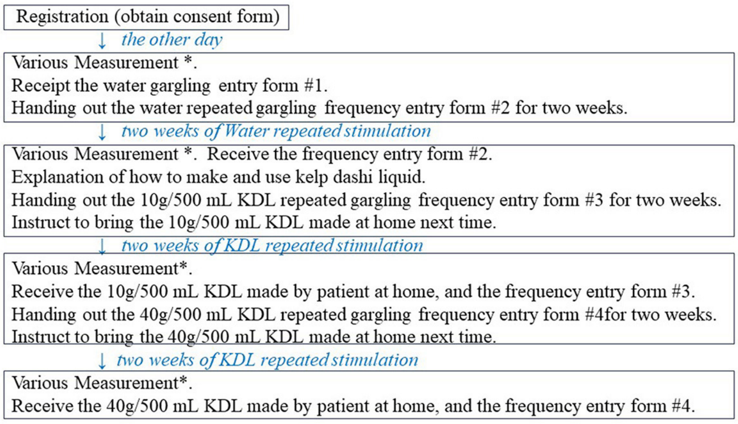

FIGURE 1
Overview of this study procedure. *Various measurements: measurements of minor salivary flow rate (MF), whole salivary flow rate (WF), recognition threshold (RT) of each of the five basic tastes, and intensity/severity of eating difficulties and other symptoms.

iv) Bitter (quinine): 0.03 mM (No. 1:Q1), 0.5 mM (No. 2: Q2), 2.5 mM
(No. 3: Q3), 12.6 mM (No. 4: Q4), and 100.7 mM (No. 5: Q5).

In this study, for each of the four basic tastes, No. 6 was used when the taste could not be determined at the concentration of No.5.

To measure the RT of umami, an aqueous solution of monosodium glutamate (MSG) was used (33), which included six concentrations of the test solution: 1 mM (No. 1:G1), 5 mM (No. 2: G2), 10 mM (No. 3:G3), 50 mM (No. 4: G4), 100 mM (No. 5:G5), and 200 mM (No. 6: G6). In this study for the umami taste, No. 7 was used when the taste could not be determined at the concentration of No. 6.

The round filter paper disc (5 mm in diameter) soaked in each taste solution was placed for 3 s on the anterior tongue. Subsequently, the paper disc was removed, and the participants were asked if they perceived any taste and to specify the type of taste they perceived. The RT was identified based on the solution number of the lowest concentration of taste solution at which the patients could correctly recognize the taste quality. The measurement was started from the lowest concentration of each taste solution until the RT was identified. To avoid interference between tastes, the patients rinsed their mouths with water several times until no previous taste remained. The order of the taste test was random except for the bitter taste test, which was performed at the end because of the unpleasantness of the taste. All patients were asked to avoid eating or drinking (except water), smoking, and brushing their teeth for at least 2 h before testing.

The diagnostic criteria of taste tests for sweet, salty, sour, and bitter are: number 1 is hypergeusia, number 2 and 3 are normal tastes, number 4 is a low degree of hypogeusia, number 5 is moderate hypogeusia, and number 6 is a high degree of hypogeusia (31).

The diagnostic criteria of the taste test for umami in the taste tests of the anterior tongue area are: number 1 is hypergeusia; number 2, 3, and 4 are normal tastes; number 5 is a low degree of hypogeusia; number 6 is moderate hypogeusia; and number 7 is a high degree of hypogeusia (33).

2.5 Measurements of the intensity/severity of eating difficulties and other symptoms

At the beginning of this experiment, the patients were asked if they had any eating difficulties and other symptoms, such as difficulty eating dry foods, difficulty in swallowing, taste impairment, continuous dry mouth sensation during the day, dry mouth sensation at night, difficulty in speaking, and burning sensation in the oral mucosa. For the assessment of the intensity/severity of eating and other difficulties, we used an originally arranged Visual Analog Scale (VAS) (34–36) that consisted of a straight line with the endpoints defining extreme limits, such as "no symptom (such as pain) at all" and "symptom (such as pain) as bad as it could be."

A line length of 10 or 15 cm showed the smallest measurement error and was most convenient for respondents, as has been previously reported (37). Therefore, we adopted a VAS of 10 cm in length. The patients were asked to mark their symptom intensity/severity level on the line between the two endpoints. The distance (mm) from the left edge (endpoint) to the site marked by the patient was defined as the VAS value.

2.6 Quantification of the amounts of various amino acids in the KDL made by patients

The KDL brought by each patient was immediately frozen and sent to the Okinawa Prefectural Health Biotechnology Research and Development Center, where the free amino acid components, such as glutamic acid, aspartic acid, serine, glycine, arginine, alanine, tyrosine, valine, phenylalanine, lysine, proline, threonine, cystine, methionine, isoleucine, leucine, tryptophan, and histidine, were analyzed and quantified. The sample was analyzed after adding three times the volume of 99.5% ethanol and filtering through a 0.2 μm filter. Highly concentrated samples were diluted with 0.1 N hydrochloric acid, filtered in the same way, and analyzed as a sample solution. The standard solution was an amino acid-mixed standard solution of type H and L-tryptophan (Wako Pure Chemical Industries, Ltd.), and the concentrations were prepared at five points in the range of 2 to 5 μmol/L and analyzed. The measurement was performed using an ultra-high-performance liquid chromatograph, the Nexere X2 series (Shimadzu Corporation, Japan), by an automatic pre-column derivation method.

2.7 Statistical analysis

The comparison of the prevalence of hypogeusis, eating difficulties, and other symptoms between the two groups was statistically calculated using Pearson's χ^2 test or [b] Fisher's exact test, depending on the variables and their normality. The Shapiro-Wilk test was used to confirm the normality. Comparisons of the changes in the salivary flow rate, in the RTs of each of the five tastes, and the intensities of the various symptoms induced before and after repeated stimulation with water or KDL were performed using the paired *t*-test. We corrected p-values to adjust multiple comparisons based on the Bonferroni correction. Spearman's rank correlation was used for the analysis of the correlation between the amount of glutamic acid in KDL and the change in the amount of saliva due to KDL stimulation. The statistical significance was set at $p<0.05$. All statistical analyses were performed using IBM's Statistical Package for the Social Sciences (SPSS), Version 29.01.

3 Results

3.1 Taste loss and saliva decrease

3.1.1 Characteristics of patients

Participants were meticulously selected for this study; seven patients withdrew due to poor physical condition, six patients dropped out without permission, and six patients used KDL prepared with an inappropriate method, resulting in the inclusion of 35 patients in the analysis. Most of the patients were women (94.2%), aged 72.31 ± 10.61 years. The MF in the lower lip was low in all patients. Patients were divided into two groups depending on whether whole saliva secretion was normal or decreased: 14 patients had a normal whole saliva secretion flow rate (NWS-LMS group, with only dry mouth sensation), and 21 patients had a low whole saliva secretion flow rate (LWS-LMS group, with hyposalivation). Table 1 shows the overall characteristics of the patients in the two groups.

No significant differences were observed in age ($p = 0.211$), sex ($p = 0.647$), number of medications used ($p = 0.654$), or frequency of smoking ($p = 0.647$) between the two groups. The prevalence of Sjögren's syndrome was significantly higher in the LWS-LMS group than in the NWS-LMS group ($p = 0.045$). There was no significant difference in the prevalence of anxiety/depression ($p = 0.782$) or diabetes ($p = 0.652$) between the two groups. The LWS-LMS group had a significantly larger proportion of patients with multiple dry mouth-related diseases mentioned above than the NWS-LMS group ($p = 0.045$). On the other hand, there was no significant difference in the rate of having non-dry mouth-related diseases, such as hypertension, kidney disease, osteoporosis, and gastroenteritis, between the two groups ($p = 0.528$).

3.1.2 Prevalence of taste loss (hypogeusia) in patients with a salivary decrease or dry mouth sensation

Table 2 shows the prevalence of taste loss (hypogeusia) in patients with hyposalivation. Umami-taste loss (hypogeusia) was notably high, affecting approximately 50% of individuals in both groups, surpassing the prevalence of other taste losses. Although hypogeusia for each of the taste qualities was more prevalent in the LWS-LMS group with hyposalivation than in the NWS-LMS group with only dry mouth sensation, there was no significant difference in the prevalence of hypogeusia for the four taste qualities:salty ($p = 0.652$), sour ($p = 0.209$), bitter ($p = 0.130$), and umami ($p = 0.581$) between the NWS-LMS and LWS-LMS groups.

3.1.3 Prevalence of eating difficulties and so on in patients with salivary decrease or dry mouth sensation

Table 3 shows the prevalence of eating difficulties and other symptoms in patients with hyposalivation, or dry mouth sensation. The rates of eating difficulties, such as difficulty in eating dry foods, difficulty in swallowing, and taste impairment, were higher in patients. There were no significant differences in each symptom between the NWS-LMS and LWS-LMS groups: dry mouth at night (NWS-LMS group with only dry mouth sensation, 84.6%; LWS-LMS group with hyposalivation, 84.2%; $p = 0.602$), difficulty in speaking (NWS-LMS group, 61.5%; LWS-LMS group, 57.9%; $p = 0.837$), difficulty in eating dry foods (NWS-LMS group, 69.2%; LWS-LMS group, 84.2%; $p = 0.281$), difficulty in swallowing (NWS-LMS group, 69.2%; LWS-LMS group, 78.9%; $p = 0.431$), burning sensation in the oral mucosa (NWS-LMS group, 84.6%; LWS-LMS group, 89.4%; $p = 0.542$), and taste impairment (NWS-LMS group, 76.9%; LWS-LMS group, 63.2%; $p = 0.335$).

3.2 Effect of repeated stimulation with the KDL gargling on saliva secretion

Although the study started with 14 and 21 patients in the NWS-LMS and LWS-LMS groups, respectively, only eight and 14 patients in the NWS-LMS and LWS-LMS groups, respectively, were able to complete 2 weeks of the three types of repeated stimulations: the first with water, the second with 10 g/500 mL KDL, and the third with 40 g/500 mL KDL.

TABLE 1 Characteristics of patients.

Characteristic	NWS-LMS group (with only dry mouth sensation) ($n = 14$)	LWS-LMS group (with hyposalivation) ($n = 21$)	p-value
Age			
Range	44–86	44–86	
Means ± SD	72.9 ± 11.65	72.00 ± 10.15	0.21[a]
	N (%)	*N* (%)	
Sex			
Female	13 (92.8)	20 (95.2)	0.647 [d]
Male	1 (8.2)	1 (4.8)	
History of systemic disease			
Sjögren's syndrome (SS)	1 (7.1)	8 (38.1)	0.045[d]
Depression/Anxiety disorder (DP/AN)	6 (42.8)	10 (47.6)	0.782[c]
Diabetes mellitus (DM)	1 (7.1)	2 (9.5)	0.652[d]
SS and/or DP/AN and/or DM	1 (7.1)	8 (38.1)	0.045[d]
Other disease (HT, CKD, BD, GID)	8 (57.1)	11 (52.3)	0.528[c]
Smoking			
Smoking	1 (7.1)	1 (4.7)	0.647[d]
No smoking	13 (92.9)	20 (95.3)	
Medication			
Range	0–10	0–10	
Number of types	4.5 ± 3.0	5.0 ± 3.3	0.654[b]

HT, hypertension; CKD, chronic kidney disease; BD, bone disease; GID, gastrointestinal disease.
p-value from [a]unpaired t-test, [b]Welch test, [c]Pearson's χ^2 test, and [d]Fisher's exact test.

TABLE 2 Prevalence of hypogeusia (taste loss) on each taste quality revealed by the taste test.

Hypogeusia	NWS-LMS group (with only dry mouth sensation group) ($n = 14$) *N* (%)	LWS-LMS group (with hyposalivation group) ($n = 21$) *N* (%)	P-value
Hypogeusia for sweet	0 (0)	5 (23.8)	
Hypogeusia for salty	1 (7.1)	2 (9.5)	0.652[b]
Hypogeusia for sour	1 (7.1)	5 (23.8)	0.209[b]
Hypogeusia for bitter	1 (7.1)	6 (28.5)	0.130[b]
Hypogeusia for umami	8 (57.1)	10 (47.6)	0.581[a]

The criteria for hypogeusia for sweet, salty, sour, and bitter are a recognition threshold of ≥4.
The criteria for hypogeusia for umami in the anterior tongue area are a recognition threshold of 7.
p-value from [a]Pearson's χ^2 test and [b]Fisher's exact test.

3.2.1 Changes in MF in the lower lip

Tables 4A, B show the changes in MF before and after repeated stimulation with water and KDL gargling.

No significant differences were observed in MF after repeated gargling with water in the two groups (NWS-LMS group, $p = 0.902$; LWS-LMS group, $p = 0.862$) (Tables 4A, B).

Repeated stimulation with gargling with 10 g/500 mL KDL for 2 weeks resulted in a significant increase in MF on the day after the stimulation ended in both groups (NWS-LMS group, $p = 0.028$; LWS-LMS group, $p = 0.029$). Moreover, significant MF increases induced by repeated gargling with 40 g/500 mL KDL were observed on the day after the stimulation ended in both groups (NWS-LMS group, $p = 0.048$; LWS-LMS group, $p = 0.011$) (Tables 4A, B).

In both the NWS-LMS and LWS-LMS groups, the MF increases after repeated gargling with 40 g/500 mL KDL were significantly greater than those with 10 g/500 mL KDL (NWS-LMS group, $p = 0.048$; LWS-LMS group, $p = 0.011$) (Figure 2).

TABLE 3 Prevalence of eating difficulties and other symptoms in patients.

Subjective symptoms present	NWS-LMS group (with only dry mouth sensation group) (n = 13) N (%)	LWS-LMS group (with hyposalivation group) (n = 19) N (%)	P-value
Continuous dry mouth sensation during the day	13 (100)	19 (100)	
Dry mouth sensation at night	11 (84.6)	16 (84.2)	0.602[b]
Difficulty in speaking	8 (61.5)	11 (57.8)	0.837[a]
Difficulty in eating dry foods	9 (69.2)	16 (84.2)	0.281[b]
Difficulty in swallowing	9 (69.2)	15 (78.9)	0.413[b]
Burning sensation in the oral mucosa	11 (84.6)	17 (89.4)	0.542[b]
Taste impairment	10 (76.9)	12 (63.1)	0.335[b]

p-value from [a]Pearson's χ^2 test and [b]Fisher's exact test.

TABLE 4A Change in the minor salivary flow rate (MF) in the NWS-LMS group induced by RG stimulation* first with water and then with kelp dashi liquid (KDL).

Minor salivary flow rates (μL/cm^2/min)	Before RG stimulation*	After RG stimulation*	Difference	P-value[†]
	Average ± SD	Average ± SD	(95% CI)	
Water (n = 8)	0.003 ± 0.004	0.005 ± 0.006	0.00 (0.00, 0.01)	0.902
10 g/500 mL KDL (n = 8)	0.005 ± 0.006	0.060 ± 0.050	0.05 (0.02, 0.09)	0.028
40 g/500 mL KDL (n = 8)	0.060 ± 0.050	2.533 ± 2.429	2.47 (0.78, 4.16)	0.048

* 2 weeks of repeated gargling stimulation.
[†] Bonferroni's corrected p-value.
Criteria for hyposalivation: MS < 1.0 μL/cm^2/min.
KDL, kelp dashi liquid; SD, standard deviation.

TABLE 4B Change in the minor salivary flow rate (MF) in the LWS-LMS group induced by RG stimulation* first with water and then with kelp dashi liquid (KDL).

Minor salivary flow rates (μL/cm^2/min)	Before RG stimulation*	After RG stimulation*	Difference	P-value[†]
	Average ± SD	Average ± SD	(95% CI)	
Water (n = 14)	0.018 ± 0.026	0.022 ± 0.042	0.00 (−0.01, 0.01)	0.862
10 g/500 mL KDL (n = 14)	0.022 ± 0.042	0.877 ± 1.134	0.86 (0.26, 1.45)	0.029
40 g/500 mL KDL (n = 14)	0.877 ± 1.134	2.345 ± 2.382	1.47 (0.60, 2.33)	0.011

* 2 weeks of repeated gargling stimulation.
[†] Bonferroni's corrected p-value.
Criteria for hyposalivation: MS < 1.0 μL/cm^2/min.
KDL, kelp dashi liquid; SD, standard deviation.

3.2.2 Changes in WF

Tables 5A, B show the changes in WF before and after repeated stimulation with water or KDL gargling. No significant differences were observed in WF after repeated gargling with water in the two groups (NWS-LMS group, $p = 1.000$; LWS-LMS group, $p = 1.000$) (Tables 5A, B).

In the NWS-LMS group, no significant changes in WF were observed after stimulation with repeated gargling with both concentrations of KDL (10 g/500 mL, $p = 0.960$; 40 g/500 mL, $p = 0.852$) (Table 5A).

In the LWS-LMS group, significant WF increases induced by repeated gargling with both concentrations of KDL were observed on the day after the stimulation ended (10 g/500 mL, $p = 0.041$; 40 g/500 mL, $p = 0.005$) (Table 5B).

In the LWS-LMS groups, the WF increases after repeated gargling with 40 g/500 mL KDL were significantly greater than those with 10 g/500 mL KDL ($p = 0.005$) (Figure 3).

There were no Sjögren's syndrome (SS) patients in the NWS-LMS group who were able to complete three consecutive stimulations; however, there were five SS out of 14 patients in the LWS-LMS group. In the LWS-LMS group, there was no difference in the amount of change in WF and MF induced by repeated stimulation with 40 g/500 mL KDL gargling between the Sjögren's syndrome (SS) patient group and the non-Sjögren's syndrome (NS) patient group. The amount of change in MF was 1.365 ±0.822 μL/cm^2/min in the SS patients group (n = 5), 1.524 ± 2.026 μL/cm^2/min in the NS patients group (n = 9); $p = 1.000$; and the amount of change in WF was 0.400 ±0.430 ml/10 min in the SS patients group (n = 5), 0.322 ± 0.327 ml/10 min in the NS patients group (n = 9); $p = 1.000$.

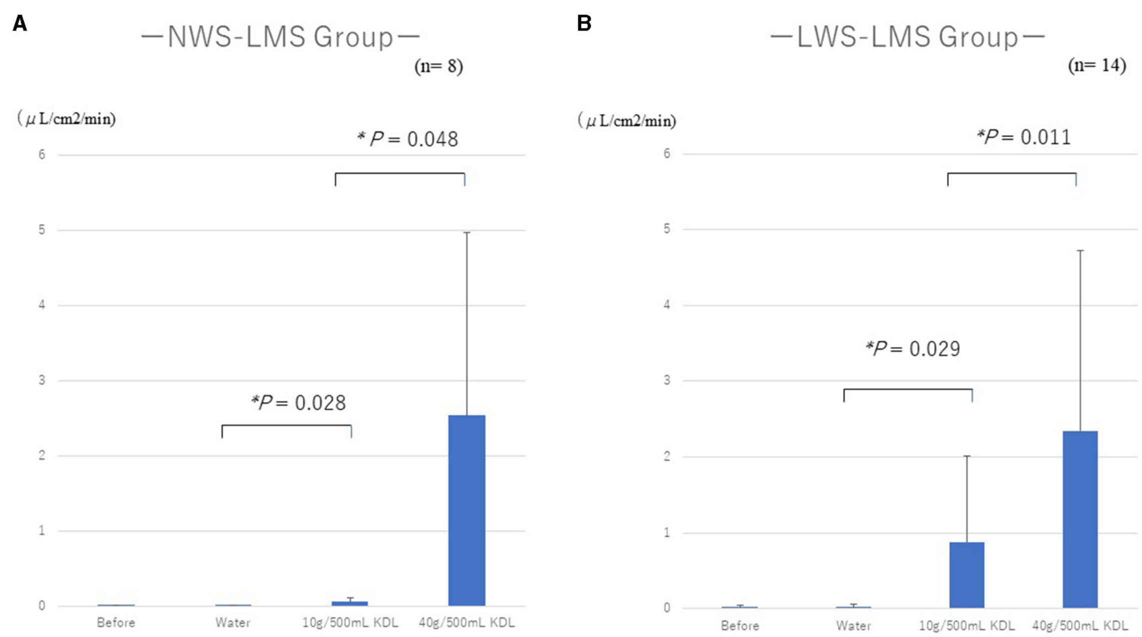

FIGURE 2
Changes in the minor salivary flow rate (MF) following repeated stimulation with water or KDL gargling in the NWS-LMS group **(A)** and LWS-LMS group **(B)** groups. KDL, kelp dashi liquid; NWS-LMS group, normal whole saliva secretion flow rate with low minor salivary gland secretion flow rate group; LWS-LMS group, low whole saliva secretion flow rate with low minor salivary gland secretion flow rate group.

TABLE 5A Change in the whole salivary flow rate (WF) in the NWS-LMS group induced by RG stimulation* first with water and then with kelp dashi liquid (KDL).

Whole salivary flow rates (mL/10 min)	Before RG stimulation* Average ± SD	After RG stimulation* Average ± SD	Difference (95% CI)	*P*-value†
Water ($n = 8$)	12.50 ± 2.71	12.45 ± 2.97	−0.05 (−0.25, 0.15)	1.000
10 g/500 mL KDL ($n = 8$)	12.45 ± 2.97	13.15 ± 3.35	0.70 (−1.14, 2.54)	0.960
40 g/500 mL KDL ($n = 8$)	13.15 ± 3.35	13.80 ± 4.50	0.65 (−0.86, 2.16)	0.852

* 2 weeks of repeated gargling stimulation.
† Bonferroni's corrected p-value.
Criteria for hyposalivation in WF < 10 mL/10 min.
KDL, kelp dashi liquid; SD, standard deviation.

TABLE 5B Change in the whole salivary flow rate (WF) in the LWS-LMS group induced by RG stimulation* first with water and then with kelp dashi liquid (KDL).

Whole salivary flow rates (mL/10 min)	Before RG stimulation* Average ± SD	After RG stimulation* Average ± SD	Difference (95% CI)	*P*-value†
Water (n = 14)	3.85 ± 2.40	3.80 ± 2.48	−0.06 (−0.26, 0.14)	1.000
10 g/500 mL KDL ($n = 14$)	3.80 ± 2.48	4.30 ± 2.92	0.50 (0.13, 0.87)	0.041
40 g/500 mL KDL ($n = 14$)	4.30 ± 2.92	4.65 ± 2.86	0.35 (0.20, 0.65)	0.005

* 2 weeks of repeated gargling stimulation.
†Bonferroni's corrected p-value.
Criteria for hyposalivation in WF < 10 mL/10 min.
KDL, kelp dashi liquid; SD, standard deviation.

3.3 Amino acids in KDL and an increase in saliva

3.3.1 Quantification of various amino acids in KDL made by each patient

The amounts of the various amino acids in the KDL made by each patient were quantitatively analyzed. Glutamic acid was the most abundant, followed by alginic acid, while other amino acids were present in very small amounts (Figure 4). The amounts of glutamic acid and other amino acids were larger in 40 g/500 mL KDL than in 10 g/500 mL KDL. There was no difference in the amount of glutamic acid in the KDL made by each patient between the NWS-LMS and LWS-LMS groups (10 g/500 mL KDL, $p = 0.428$; 40 g/500 mL KDL, $p = 0.745$) (Table 6).

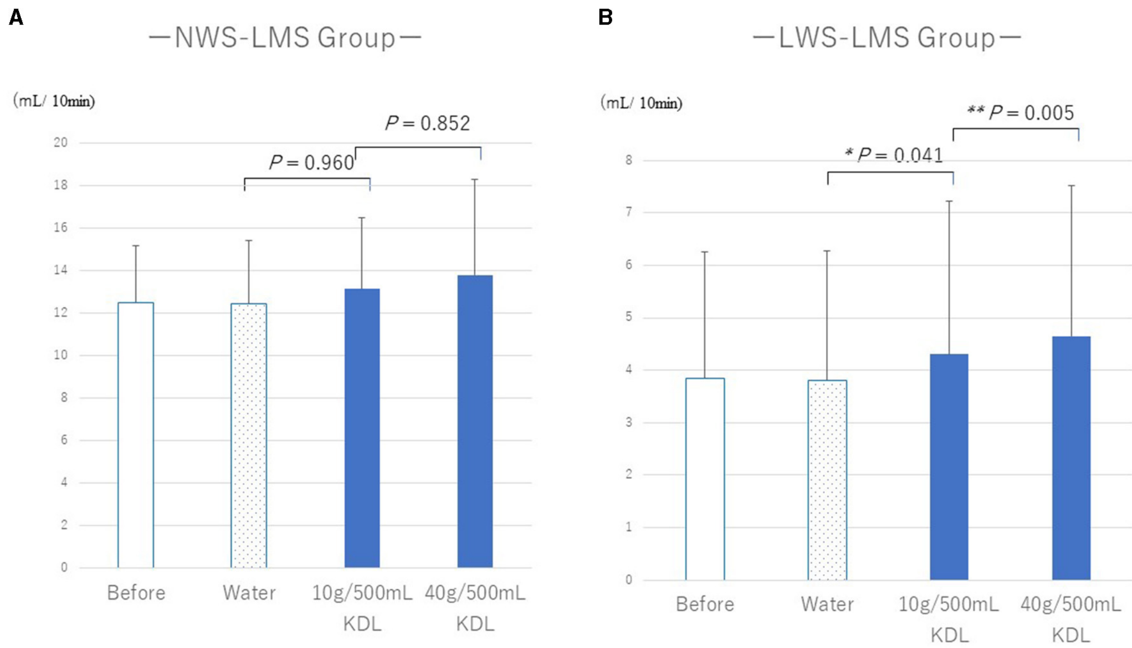

FIGURE 3
Changes in the whole salivary flow rate (WF) following repeated gargling stimulation with water or KDL gargling in the NWS-LMS **(A)** and LWS-LMS **(B)** groups. KDL, kelp dashi liquid; NWS-LMS group, normal whole saliva secretion flow rate with low minor salivary gland secretion flow rate group; LWS-LMS group, low whole saliva secretion flow rate with low minor salivary gland secretion flow rate group.

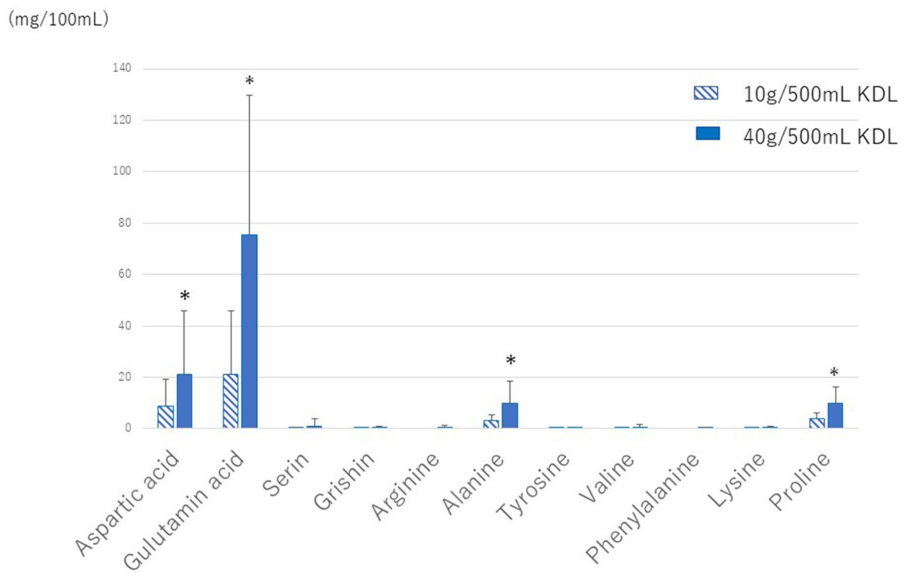

FIGURE 4
Amounts of various amino acids in KDL made by each patient. KDL, kelp dashi liquid. * 2 weeks of repeated gargling stimulation.

3.3.2 Correlation between the changes in MF or WF and the amount of glutamic acid

Spearman's correlation analysis showed that the increase in MF following repeated stimulation with KDL gargling was correlated with the amount of glutamic acid in KDL in both groups (NWS-LMS group, r = 0.711, $p < 0.01$; LWS-LMS group, r = 0.490, $p < 0.01$). On the other hand, the increase in WF following repeated stimulation with KDL gargling was not correlated with the amount of glutamic acid in KDL in both groups (NWS-LMS group, r = 0.361; LWS-LMS group, r = 0.072).

TABLE 6 Amount of glutamic acid in kelp dashi liquid (KDL) made by patients.

Glutamic acid (mg /100 mL)	NWS-LMS group Average ± SD	LWS-LMS group Average ± SD	*P*-value
10 g/500 mL KDL	25.56 ± 14.71	32.73 ± 26.83	0.428[b]
40 g/500 mL KDL	81.27 ± 43.91	76.33 ± 36.16	0.745[a]

p-value from [a]unpaired t-test and [b]Welch test.
KDL, Kelp dashi liquid; SD, standard deviation.

TABLE 7A Change in the recognition threshold (RT) of each taste in the NWS-LMS group induced by RG stimulation* with water and with kelp dashi liquid (KDL).

Recognition threshold (RT)	Before RG stimulation* Average ± SD	After RG stimulation* Average ± SD	Difference (95% CI)	*P*-value†
Sweet	($n = 8$)	($n = 8$)		
Water	2.7 ± 0.5	2,6 ± 0.5	−0.13 (−0.37, 0.12)	1.000
10 g/500 mL KDL	2.6 ± 0.5	2.3 ± 0.5	−0.38 (−0.72, −0.02)	0.398
40 g/500 mL KDL	2.3 ± 0.5	1.9 ± 0.7	−0.38 (−0.72, −0.02)	0.398
Salty	($n = 8$)	($n = 8$)		
Water	2.5 ± 0.8	2.4 ± 0.7	−0.13 (−0.37, 0.12)	1.000
10 g/500 mL KDL	2.4 ± 0.7	2.3 ± 0.5	−0.13 (−0.37, 0.12)	1.000
40 g/500 mL KDL	2.3 ± 0.5	1.9 ± 0.7	−0.38 (−0.89, 0.14)	0.985
Sour	($n = 8$)	($n = 8$)		
Water	2.7 ± 0.7	2.6 ± 0.7	−0.13 (−0.37, 0.12)	1.000
10 g/500 mL KDL	2.6 ± 0.7	2,6 ± 0.8	0.00 (0.00, 0.00)	1.000
40 g/500 mL KDL	2.6 ± 0.8	2.3 ± 0.5	−0.25 (−0.57, 0.07)	0.852
Bitter	($n = 8$)	($n = 8$)		
Water	2.7 ± 1.0	2.6 ± 0.8	−0.13 (−0.57, 0.32)	1.000
10 g/500 mL KDL	2.6 ± 0.8	2.5 ± 0.7	−0.13 (−0.37, 0.12)	1.000
40 g/500 mL KDL	2.5 ± 0.7	2.1 ± 0.4	−0.38 (−0.73, −0.02)	0.398
Umami	(n =8)	($n = 8$)		
Water	6.5 ± 0.7	6.3 ± 0.8	−0.13 (−0.37, 0.12)	1.000
10 g/500 mL KDL	6.3 ± 0.8	4.7 ± 0.9	−1.63 (−1.98, −1.27)	<0.0001
40g/500 mL KDL	4.7 ± 0.9	2.4 ± 0.8	−2.38 (−2.73, −2.02)	<0.0001

* 2 weeks of repeated gargling stimulation.
Criteria for hypogeusia for sweet, salty, sour, and bitter: recognition threshold (RT) ≥4.
Criteria for hypogeusia for umami in the anterior tongue area: recognition threshold (RT) number of 7.
† Bonferroni's corrected p-value.
KDL, kelp dashi liquid; SD, standard deviation.

3.4 Effects of repeated stimulation with KDL gargling on the RTs of the five basic tastes

The repeated stimulation with water gargling did not change the RT in both groups.

Repeated stimulation with KDL gargling significantly reduced the RT for umami, returning the value to normal in both groups: NWS-LMS group: 10 g/500 mL KDL, $p < 0.0001$; 40 g/500 mL KDL, $p < 0.0001$ (Table 7A); LWS-LMS group: 10 g/500 mL KDL, $p < 0.0001$; 40 g/500 mL KDL, $p < 0.0001$ (Table 7B).

In the NWS-LMS group, there was no difference in the RT of the four basic tastes, i.e., sweet, salty, sour, and bitter, with almost no hypogeusia except for the umami taste (Table 7A).

In the LWS-LMS group, repeated stimulation with 40 g/500 mL KDL gargling significantly reduced each taste's RT (sweet: 10 g/500 mL KDL, $p = 0.093$; 40 g/500 mL KDL, $p = 0.002$, salty: 10 g/500 mL KDL, $p = 0.947$; 40 g/500 mL KDL, $p = 0.016$, sour: 10 g/500 mL KDL, $p = 0.093$; 40 g/500 mL KDL, $p = 0.016$, bitter: 10 g/500 mL KDL, $p = 0.093$; 40 g/500 mL KDL, $p = 0.040$) (Table 7B).

TABLE 7B Change in the recognition threshold (RT) of each taste in the LWS-LMS group induced by RG stimulation* with water and with kelp dashi liquid (KDL.

Recognition threshold (RT)	Before RG stimulation* Average ± SD	After RG stimulation* Average ± SD	Difference (95% CI)	P-value†
Sweet	($n = 14$)	($n = 14$)		
Water	3.1 ± 0.8	3.0 ± 0.9	−0.07 (−0.22, 0.07)	1.000
10 g/500 mL KDL	3.0 ± 0.9	2.7 ± 0.5	−0.36 (−0.62, −0.10)	0.093
40 g/500 mL KDL	2.7 ± 0.5	2.1 ± 0.3	−0.64 (−0.90, −0.38)	0.002
Salty	($n = 14$)	($n = 14$)		
Water	2.5 ± 0.7	2.4 ± 0.6	−0.14 (−0.33, 0.05)	0.824
10 g/500 mL KDL	2.4 ± 0.6	2.2 ± 0.6	−0.21 (−0.52, 0.09)	0.947
40 g/500 mL KDL	2.2 ± 0.6	1.7 ± 0.5	−0.50 (−0.77, −0.23)	0.016
Sour	($n = 14$)	($n = 14$)		
Water	3.0 ± 0.8	2.9 ± 0.7	−0.07 (−0.32, 0.18)	1.000
10 g/500 mL KDL	2.9 ± 0.7	2,6 ± 0.5	−0.36 (−0.62, −0.10)	0.093
40 g/500 mL KDL	2.6 ± 0.5	2.1 ± 0.3	−0.50 (−0.77, −0.23)	0.016
Bitter	(n =14)	($n = 14$)		
Water	3.0 ± 0.8	2.9 ± 0.7	−0.07 (−0.21, 0.07)	1.000
10 g/500 mL KDL	2.9 ± 0.7	2.6 ± 0.5	−0.36 (−0.62, −0.10)	0.093
40 g/500 mL KDL	2.6 ± 0.5	2.1 ± 0.4	−0.43 (−0.70, −0.16)	0.040
Umami	(n =14)	($n = 14$)		
Water	6.0 ± 1.2	6.0 ± 1.1	0.00 (−0.21, 0.21)	1.000
10 g/500 mL KDL	6.0 ± 1.1	4.7 ± 1.1	−1.29 (−1.67, −0.91)	<0.0001
40 g/500 mL KDL	4.7 ± 1.1	3.1 ± 0.8	−1.57 (−2.10, −1.04)	<0.0001

* 2 weeks of repeated gargling stimulation.
Criteria for hypogeusia for sweet, salty, sour, and bitter: recognition threshold (RT) ≥4.
Criteria for hypogeusia for umami in the anterior tongue area: recognition threshold (RT) number of 7.
† Bonferroni's corrected p-value.
KDL, kelp dashi liquid; SD, standard deviation.

3.5 Effects of repeated stimulation with KDL gargling on eating difficulties and other symptoms

Depending on the concentration of KDL, repeated stimulation with gargling alleviated eating difficulties and other symptoms. No significant difference was observed in the dry mouth-related symptoms after repeated stimulation with water gargling.

Continuous dry mouth sensation during the day was reduced by repeated stimulation with KDL in both groups: NWS-LMS group: 10 g/500 mL KDL, $p = 0.050$; 40 g/500 mL KDL, $p < 0.001$ (Table 8A); LWS-LMS group: 10 g/500 mL KDL, $p = 0.009$; 40 g/500 mL KDL, $p = 0.011$ (Table 8B).

In the NWS-LMS group, repeated stimulation with KDL gargling significantly reduced below-subject symptoms: difficulty in speaking: $p = 0.019$,10 g/500 mL KDL; $p = 0.035$, 40 g/500 mL KDL; difficulty in eating dry foods: $p = 0.010$, 40 g/500 mL KDL; difficulty in swallowing: $p = 0.049$, 40 g/500 mL KDL; burning sensation in the oral mucosa: $p = 0.042$, 40 g/500 mL KDL; taste impairment: $p = 0.009$, 40 g/500 mL KDL) (Table 8A).

In the LWS-LMS group, repeated stimulation with KDL gargling significantly reduced below-subject symptoms: difficulty in speaking: $p = 0.014$, 40 g/500 mL KDL; difficulty in eating dry foods: $p = 0.0042$, 40 g/500 mL KDL; difficulty in swallowing: p =0.002, 40 g/500 mL KDL; burning sensation in the oral mouth: p =0.002,10 g/500 mL KDL; p =0.020, 40 g/500 mL KDL; taste impairment: p =0.007,10 g/500 mL KDL; p =0.002, 40 g/500 mL KDL) (Table 8B). There was no significant difference in dry mouth sensation at night by repeated simulation with KDL in both groups.

Table 9 shows a difference in the effects of KDL stimulation on each subject's symptoms between the two groups. Improvement of VAS values with KDL stimulation for difficulty in speaking (p =0.033, 10 g/500 mL KDL; p =0.015, 40 g/500 mL KDL), and difficulty in eating dry foods (p =0.026, 40 g/500 mL KDL) were more significant in the NWS-LMS group than in the LWS-LMS group (Table 9).

4 Discussion

This study revealed that the umami taste in KDL might contribute to the maintenance of the normal taste sensation and

TABLE 8A Change in the intensity/severity (VAS) of subject symptoms in the NWS-LMS group. Induced by RG stimulation* with water and with kelp dashi liquid (KDL).

Intensity/severity (VAS) after RG stimulation*	Before RG stimulation*	After RG stimulation*	Difference	*P*-value†
	Average ± SD	Average ± SD	(95% CI)	
Continuous dry mouth feeling during the day	($n = 8$)	($n = 8$)		
Water -	100	96.8 ± 4.5	–	-
10 g/500 mL KDL	96.8 ± 4.5	70.6 ± 18.9	−26.3 (−40.0, −12.6)	0.050
40 g/500 mL KDL	70.6 ± 18.9	32.7 ± 16.1	−37.9 (−45.6, −30.1)	<0.001
Dry mouth feeling at night	($n = 8$)	($n = 8$)		
Water	100	100	–	-
10 g/500 mL KDL	100	91.4 ±18.6	−8.6 (−21.6, 4.3)	1.000
40 g/500 mL KDL	91.4 ±18.6	46.2 ±38.5	−45.1 (−70.4, −19.9)	0.069
Difficulty in speaking	($n = 8$)	($n = 8$)		
Water	100	100	–	-
10 g/500 mL KDL	100	72.5 ±17.1	−27.5 (−39.4, −15.6)	0.019
40 g/500 mL KDL	72.5 ±17.1	37.5 ± 17.5	−35.0 (−52.0, −18.0)	0.035
Difficulty in eating dry foods	($n = 7$)	($n = 7$)		
Water	100	100	–	-
10 g/500 mL KDL	100	90.0 ± 8.1	−10.0 (−16.1, −4.0)	0.124
40 g/500 mL KDL	90.0 ± 8.1	31.4 ± 22.7	−58.6 (−79.3, −37.9)	0.010
Difficulty in swallowing	(n = 8)	(n = 8)		
Water	100	98.9 ± 3.3	–	-
10 g/500 mL KDL	98.9 ± 3.3	72.5 ± 35.9	−26.4 (−52.0, −0.8)	0.584
40 g/500 mL KDL	72.5 ± 35.9	36.2 ± 16.0	−36.3 (−55.1, −17.4)	0.049
Burning sensation of oral mucosa	($n = 8$)	($n = 8$)		
Water	100	100	–	-
10 g/500 mL KDL	100	60.0 ± 33.5	−40.0 (−63.2, −16.8)	0.082
40 g/500 mL KDL	60.0 ± 33.5	27.5 ± 24.4	−32.5 (−48.9, −16.1)	0.042
Taste impairment	($n = 8$)	($n = 8$)		
Water	100	98.0 ± 6.3	–	-
10 g/500 mL KDL	98.0 ± 6.3	69.0 ± 28.3	−29.0 (−48.9, −9.1)	0.171
40 g/500 mL KDL	69.0 ± 28.3	13.3 ± 15.0	−55.8 (−77.0, −34.5)	0.009

* 2 weeks of repeated gargling stimulation.
† Bonferroni's corrected p-value.
KDL, kelp dashi liquid; SD, standard deviation.

promote healthy eating habits in older adults. First, repeated stimulation with KDL improved taste decline and maintained the normal taste sensation by increasing saliva production in MF and WF in older adults through the umami-taste gustatory reflex. Second, repeated stimulation with KDL alleviated subjective taste impairment and subjective eating and swallowing difficulties.

In this study, the incidence of taste loss in the patients with hyposalivation (LWS-LMS group) was 9.5% for the salty taste, approximately 24% for the sweet and sour taste, and 28.5% for the bitter taste. However, umami-taste loss was the most prevalent, with an incidence of 48% occurring in approximately half of the patients. In the patients with only oral dryness (NWS-LMS group), the incidence of umami-taste loss was 57%, which is similar to that in the patients with hyposalivation patients, although it was 7% for the salty, sour, and bitter tastes. Since taste sensation is associated with WF, hyposalivation, which involves decreased WF, is known to be the most common cause of taste loss in older adults (5). However, the relationship between taste loss and decreased MF has not yet been established. Our results revealed that umami-taste loss is more likely to occur in older people with dry mouths than taste loss of the other four basic taste qualities. Moreover, in particular, umami-taste loss was also observed in older adults with decreased

TABLE 8B Changes in the intensity/severity (VAS) of subject symptoms in the LWS-LMS group. Induced by RG stimulation* with water and with kelp dashi liquid (KDL).

Intensity/severity (VAS) after RG stimulation*	Before RG stimulation*	After RG stimulation*	Difference	P-value†
	Average ± SD	Average ± SD	(95% CI)	
Continuous dry mouth feeling during the day	($n = 14$)	($n = 14$)		
Water	100	98.1 ± 3.8	–	-
10 g/500 mL KDL	98.1 ± 3.8	76.4 ± 20.1	−21.7 (−32.1, −11.3)	0.009
40 g/500 mL KDL	76.4 ± 20.1	55.3 ± 22.2	−21.1 (−32.4, −9.9)	0.011
Dry mouth feeling at night	($n = 9$)	($n = 9$)		
Water	100	100	–	-
10 g/500 mL KDL	100	85.0 ±16.9	−15.0 (−26.1, −3.9)	0.204
40 g/500 mL KDL 85.0 ±16.9		82.2 ±18.5	−2.8 (−15.5, 9.9)	1.000
Difficulty in speaking	(n = 11)	(n = 11)		
Water	100	100	–	-
10 g/500 mL KDL	100	96.7 ± 7.1	−3.3 (−7.5, 0.9)	1.000
40 g/500 mL KDL	96.7 ± 7.1	70.9 ± 22.5	−25.8 (−38.0, −13.6)	0.014
Difficulty in eating dry foods	($n = 12$)	($n = 12$)		
Water	100	100	–	-
10 g/500 mL KDL	100	85.8 ± 17.8	−14.2 (−24.3, −4.1)	0.131
40 g/500 mL KDL	85.8 ± 17.8	71.0 ± 26.5	−14.8 (−23.3, −6.2)	0.042
Difficulty in swallowing	($n = 14$)	($n = 14$)		
Water	100	100	-	-
10 g/500 mL KDL	100	84.1 ± 21.1	−15.9 (−26.9, −4.8)	0.104
40 g/500 mL KDL	84.1 ± 21.1	61.4 ± 30.3	−22.7 (−31.6, −13.8)	0.002
Burning sensation of oral mucosa	($n = 14$)	($n = 14$)		
Water	100	99.4 ± 2.4	-	-
10 g/500 mL KDL	99.4 ± 2.4	58.6 ± 32.3	−40.9 (−57.4, −24.3)	0.002
40 g/500 mL KDL	58.6 ± 32.3	32.8 ± 28.5	−25.8 (−39.6, −12.0)	0.020
Taste impairment	($n = 12$)	($n = 12$)		
Water	100	99.1 ± 2.9	-	-
10 g/500 mL KDL	99.1 ± 2.9	58.9 ± 30.2	−40.3 (−57.9, −22.6,)	0.007
40 g/500 mL KDL	58.9 ± 30.2	35.0 ± 26.5	−23.9 (−32.6, −15.2)	0.002

* 2 weeks of repeated gargling stimulation.
† Bonferroni's corrected p-value.
KDL, kelp dashi liquid; SD, standard deviation.

MF and normal WF, which was similar to the finding in patients with hyposalivation. This suggests that umami-taste loss might be more dependent on the decreased MF than on WF.

In this study, repeated stimulation with KDL normalized RTs, improved taste loss for all tastes, including umami-taste, and sustained normal taste sensation in both patients with hyposalivation (LWS-LMS group) and dry mouth sensation (NWS-LMS group).

Umami-taste loss in older people has been reported to be associated with loss of appetite and weight loss. It has been reported that umami-taste loss was observed in 16% of older patients with hypogeusia, who experienced decreased appetite along with weight loss and poor physical condition (38). The umami sensitivity in these patients improved through the treatment, including therapy for increasing saliva; thereafter, these patients regained their appetite and weight, as well as their health (38). In general, dry mouth which causes taste decline and leads to frailty, occurs frequently in older adults. It has also been reported that dry mouth (xerostomia) in older adults is linked to a decline in physical function (39) and the progression of frailty later in life (40). Considering our present findings, it is possible that taste loss caused by dry mouth might also contribute to the onset

TABLE 9 Comparison of intensity/severity (VAS) change of subjective symptoms by RG stimulation* between NWS-LMS and LWS-LMS groups.

Intensity/severity (VAS) after RG stimulation*	NWS-LMS group	LWS-LMS group	Difference	*P*-value†
	Average ± SD	Average ± SD	(95% CI)	
Continuous dry mouth feeling during the day	($n = 8$)	($n = 14$)		
Water	96.8 ± 4.5	98.1 ± 3.8	–	-
10 g/500 mL KDL	70.6 ±18.9	76.4 ±20.1	5.8 (−11.1, 22.7)	1.000
40 g/500 mL KDL	32.7 ±16.1	55.3 ±22.2	22.5 (5.8, 39.3)	0.091
Dry mouth feeling at night	($n = 8$)	($n = 9$)		
Water	100	100	–	-
10 g/500 mL KDL	91.4 ±18.6	85.0 ±16.9	−6.4 (−23.4, 10.7)	1.000
40 g/500 mL KDL	46.2 ±38.5	82.2 ±18.5	36.0 (6.7, 65.3)	0.262
Difficulty in speaking	($n = 8$)	($n = 11$)		
Water	100	100	–	-
10 g/500 mL KDL	72.5 ±17.1	96.7 ± 7.1	24.2 (11.6, 36.8)	0.033
40 g/500 mL KDL	37.5 ±17.5	70.9 ±22.5	33.4 (15.4, 51.4)	0.015
Difficulty in eating dry foods	($n = 7$)	($n = 12$)		
Water	100	100	–	-
10 g/500 mL KDL	90.0 ± 8.1	85.8 ±17.8	−4.2 (−15.9, 7.6)	1.000
40 g/500 mL KDL	31.4 ±22.7	71.0 ±26.5	39.7 (18.0, 61.4)	0.026
Difficulty in swallowing	($n = 8$)	($n = 14$)		
Water	98.9 ± 3.3	100	–	-
10 g/500 mL KDL	72.5 ±35.9	84.1 ±21.1	11.6 (−15.6, 38.9)	1.000
40 g/500 mL KDL	36.2 ±16.0	61.4 ±30.3	25.2 (5.8, 44.6)	0.134
Burning sensation of oral mucosa	($n = 8$)	($n = 14$)		
Water	100	99.4 ± 2.4	–	-
10 g/500 mL KDL	60.0 ±33.5	58.6 ±32.3	−1.4 (−30.2, 27.3)	1.000
40 g/500 mL KDL	27.5 ±24.4	32.8 ±28.5	5.3 (−17.3, 27.9)	1.000
Taste impairment	($n = 8$)	($n = 12$)		
Water	98.0 ± 6.3	99.1 ± 2.9	–	-
10 g/500 mL KDL	69.0 ± 28.3	58.9 ± 30.2	−10.1 (−36.1, 15.9)	1.000
40 g/500 mL KDL	13.3 ± 15.0	35.0 ± 26.5	21.8 (3.5, 40.0)	0.219

* 2 weeks of repeated gargling stimulation.
† Bonferroni's corrected p-value.
KDL, kelp dashi liquid; SD, standard deviation.

of frailty in older adults. Taste decline in older adults is less noticeable than vision and hearing loss (41); it is difficult to detect because it occurs gradually. This study shows that umami-taste loss also generally occurs in those who only have dry mouth sensations. Repeated stimulation with KDL gargling could improve and sustainably maintain the taste sensation, especially umami-taste, and lead to maintain their appetite and weight, and it is thought to be effective in preventing frailty in older adults.

Since decreased saliva is a common cause of taste decline in older adults (6, 7), it is necessary to sustain an increase in saliva over a long period of time to improve and maintain the taste sensation. However, it has still been difficult to sustainably increase saliva production to improve taste because older adults are more likely to experience adverse effects caused by medications. The taste-salivary reflex has been studied in an attempt to use it as a remedy to increase saliva production. To date, it has been reported that after an increase induced by a moisturizing gel containing sweet and sour substances, whole saliva returned to normal levels in participants with oral dryness after 10 min (42). In this study, we used umami-rich KDL as a stimulus to induce the umami-taste salivary reflex. Repeated stimulation with KDL increased the decreased MF and WF until the next day after stimulation, implying that the effects lasted at least half a day, the longest period to date. The current results reveal that KDL is effective in continuously increasing saliva production.

KDL contains glutamic acid, which imparts the umami-taste sensation. The KDL made by each patient contained significantly more glutamic acid than other amino acids. As the amount of kelp increased, the glutamic acid in the KDL also increased. The amount of glutamic acid affected the decrease in RT for each taste quality as well as the increase in MF and WF. In other words, it was dependent on the intensity of the umami flavor. We previously reported that in healthy subjects with an average age of 31 years old, the increase in MF induced by the umami taste lasted for more than 25 min after the stimulation ended, whereas the increase in MF induced by the other four basic tastes (sweet, salty, sour, and bitter) returned to the baseline level quickly (24). Moreover, in a comparative study with similar intensities of each of the five taste solutions, the umami-taste increased the MF in the lower, similar to the sour state and significantly more than the sweet, salty, and bitter tastes (25) in healthy volunteers with an average age of 31 years old (31, 33). Considering these previous studies, the umami taste of KDL might have caused a long-lasting increase in MF and WF, even in older patients with xerostomia. The useful effects of KDL on the taste sensation would appear to be based on this finding.

The mechanism behind the long-lasting increase in MF and WF induced by KDL stimulation may depend on the umami aftertaste. The duration of the aftertaste differs depending on each taste quality (43). The sour aftertaste elicited by tartaric acid decreases rapidly after spitting it out, but the umami aftertaste elicited by glutamic acid remains strong and persists even after spitting it out. Umami has unique temporal characteristics, i.e., a long-lasting aftertaste (26).

In this study, the improvement in taste decline induced by KDL stimulation was particularly remarkable in the case of the umami taste. It has been reported that the expression of taste-related genes in the tongue, induced by tongue stimulation with MSG, increased the expression levels of the umami taste-related genes *T1R1* and *T1R3* (44). It is possible that in this study, repeated stimulation with KDL increased the expression of umami taste-related genes *T1R1* and *T1R3* in the tongue, resulting in increased umami sensitivity and improved umami-taste loss.

Older individuals often complain of eating difficulties, including difficulty in swallowing and chewing. In this study, we demonstrated that repeated stimulation with KDL alleviated the subjective difficulty in swallowing and eating dry food not only in older individuals with hyposalivation (LWS-LMS group) but also in those with only dry mouth sensation (NWS-LMS group). In addition, repeated stimulation with KDL increased MF in both groups. These results showed that these subjective eating difficulties occurred when MF was decreased, even if WF was normal. The amount of saliva secreted by the minor salivary glands is extremely small (8% of whole saliva); however, these subjective symptoms might be alleviated by increasing MF via the umami-taste salivary reflex. Until now, subjective difficulty in swallowing and eating has been associated with whole saliva (14, 45), and the relationship with minor gland saliva remains unclear.

In this study, we demonstrated that decreased MF is associated with subjective dry mouth sensations. Previous reports have shown that patients with complaints of dry mouth are less likely to have high MF (46) and that individuals with normal stimulated and resting whole salivary secretion who complain of xerostomia both in the day and night have significantly lower MF (47). Moreover, patients with dry mouth have significantly lower MF than WF compared to healthy subjects (28). In the present study, these KDL-related improvements in subjective eating difficulties were associated with increased MF. Minor gland saliva contains large amounts of mucin and secretory IgA, which contribute to moisturization and are involved in the maintenance of oral mucosal health (17–19). Remarkably, major salivary glands secrete saliva when stimulated, whereas minor gland saliva is constantly secreted, both when stimulated and at rest (15).

The present study is the first to demonstrate that subjective eating difficulties in older individuals occur when MF is decreased, even if WF is normal, and that KDL stimulation alleviates these subjective eating difficulties. KDL improves eating difficulties by increasing MF via the umami-taste salivary reflex, and it could potentially promote healthy daily dietary habits.

Complaints of burning sensation in the oral mucosa are common among oral problems in older individuals. In this study, oral mucosal burning sensation was one of the most common complaints and was observed in more than 80% of patients with decreased MF, regardless of WF, and was improved by repeated stimulation with KDL. Organic abnormalities, such as wounds in the oral mucosa, were not observed in the study participants. In the absence of organic abnormalities, a burning sensation in the oral mucosa that lasts for more than 2 h a day for more than 3 months is defined as burning mouth syndrome (BMS) (48). BMS often occurs in older and middle-aged women and, owing to poorly understood etiologies, is difficult to treat (49). A burning sensation in the oral mucosa is associated with various accompanying symptoms, among which dry mouth has been reported to be the most common (50). The reduction in the burning sensation in the oral mucosa observed in this study might be due to the KDL-induced MF increase. Therefore, KDL may also be beneficial in the treatment of various oral problems that are associated with aging and may promote a healthy daily lifestyle in older individuals.

Even for healthy people, habitually gargling with KDL could have the effect of preventing each oral function, particularly the difficulty of swallowing and eating and the burning sensation in the oral mucosa, which are common problems in older individuals.

This study had several limitations. First, this was a single-center study conducted at a dental department in a Japanese university hospital, which included Japanese people who are familiar with kelp dashi; therefore, the generalizability of the findings to diverse domestic and international populations or settings may be restricted. Research subjects from countries other than Japan and/or subjects of non-Japanese ethnicity who are not familiar with KDL are needed. Second, only a few men were included in this study, and sex differences were not investigated. Sex differences in KDL effects need to be studied. Although this study included a small number of patients, the differences in the effects of KDL, including the improvement in the RT of the taste qualities and increased saliva secretion, were significant. The reason for the small number of patients was that the study setting involved a long-term, complex method cycle that combined different stimulation methods every 2 weeks.

In the future, we plan to verify the KDL stimulation effects by increasing the number of participants using a simplified method and to report any new developments. Third, in this study that confirms the potential of KDL umami in older patients with xerostomia, subjects made fresh KDL regularly every 2 days at home using a common recipe that we provided them. Since KDL is rich in amino acids, which are nutrients that promote the growth of microorganisms, it spoils easily. We would like to develop the utility of the KDL effects into further therapeutic development research, in which it would be necessary to provide the subjects with homogenized KDL containing useful components. In the future studies, it is necessary to have comparative experiments between a group stimulated with KDL containing useful ingredients, a group stimulated with only glutamic solution, and a group stimulated with other taste solutions, such as glucose solution, to clarify the usefulness of KDL umami. Fourth, the relationship between age-related structural changes or damage, such as Sjögren's syndrome, in the minor salivary glands is unclear. Although the effects of aging on the minor salivary glands, such as structural degenerative changes, have been reported (51), salivary flow measurements in older people showed both decreased and unchanged function of the minor salivary glands (52–54). The relationship between sex, aging, diseases, and MF is yet to be conclusively clarified. In this study, we found that KDL repeated stimulation increased MF and WF both in the patients with Sjögren's syndrome and in the other patients. Various therapeutic options, including medications, transcutaneous electrical stimulation, and acupuncture, have been used to treat the dry mouth caused by Sjögren's syndrome. However, the available therapeutic options provide only limited benefits for these patients (55). KDL may promote the MF and the WF in patients with or without Sjögren's syndrome. Since the number of cases was small in this study, further research that increases the number of cases is needed. Furthermore, this study targeted the lower lip minor salivary glands, and differences in responses have been shown depending on the location of the minor salivary glands (18, 54). Therefore, it is also necessary to examine the different effects of KDL based on different locations of the minor salivary glands.

In conclusion, repeated stimulation with KDL improved taste decline and maintained normal taste sensation by increasing saliva production, as measured by MF and WF, in older adults through the umami-taste salivary reflex. Moreover, repeated stimulation with KDL alleviated subjective taste impairment, subjective eating difficulty, subjective swallowing difficulty, and burning sensations in the oral mucosa, all of which are daily eating and oral problems that plague older adults.

This study is the first to report these findings that emphasize the usefulness and contribution of KDL umami in improving taste decline, sustainably maintaining normal taste sensation, and promoting healthy eating habits in older adults.

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

This study was approved by the Ethics Committee of the Tohoku University Graduate School of Dentistry (Ethics No. 2016-3-027). 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

SS-K: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Visualization, Writing – original draft, Writing – review & editing. SG: Methodology, Validation, Writing – review & editing. NS: Funding acquisition, Investigation, Formal analysis, Writing – review & editing. HU: Resources, Writing – review & editing. MK: Methodology, Supervision, Writing – review & editing.

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Conflict of interest

HU was employed by Ajinomoto Co., Inc.

The remaining 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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Initial implicit association between whole grains and taste does not predict consumption of whole grains in low-whole grain consumers: a pilot randomized controlled trial

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Background: Health benefits of whole grain (WG) consumption are well documented. Current Dietary Guidelines for Americans recommend at least half of total grains consumed be WG; however, Americans consume less than one serving of WG per day. Inferior taste of whole grain products as compared with refined grain products has been reported as one of the main barriers to acceptability and consumption of whole grains. In this pilot study, we aimed to determine if mere exposure to WG foods in self-reported low WG consumers would improve their implicit associations between WG and pleasant taste.

Methods: Healthy adults (n=45) were provided a variety of WG or refined grain (RG) products for home use for 6 weeks. Intake was measured by calculating disappearance and verified by a daily log. At the beginning and end of the intervention, we administered an Implicit Association Test (IAT), a computer test designed to measure indirectly the strength of association between pairs of concepts: (a) two contrasted target categories (WG and RG food images) and (b) two contrasted attribute categories (words relating to pleasant or unpleasant taste) via a classification task. Response time was used to calculate IAT D scores, indicating the strength of implicit associations between WG and RG and positive or negative taste.

Results: ANCOVA showed that average D scores at the end of the study shifted significantly toward a positive implicit association between WG and good taste (p<0.05) in participants whose baseline D scores indicated an initial preference for RG over WG. No significant differences were found between the WG and RG groups in overall consumption of provided grain products.

Conclusion: These findings suggest that mere exposure to WG products over an extended period of time in a free-living situation can improve automatic attitudes toward WG, potentially leading to increased consumption of WG foods.

Clinical trial registration: Clinicaltrials.Gov, identifier NCT01403857.

KEYWORDS

implicit association, Implicit Association Test, mere exposure, taste, whole grain consumption, whole grains

1 Introduction

The health benefits of whole grain (WG) consumption are well documented (1–3). Whole grain consumption has been associated with reduced risk of cardiovascular disease (4, 5), type 2 diabetes (6, 7), inflammation (8), and certain cancers (9–12). In addition, large prospective studies of the US population found higher whole grain consumption associated with lower total mortality (13–15). Accordingly, since 2005, the Dietary Guidelines for Americans (DGA), recommend that individuals consume ≥3 ounce-equivalents/servings of WG/day or that at least half of total grains consumed be WG (16). The food industry has responded to these guidelines with a dramatic increase in the production and marketing of WG products, along with efforts to assist the consumer in identifying WG products at the point of purchase (17, 18).

Despite increased variety, availability, and promotion of WG products, consumption of WG in the US remains low, with Americans in all age groups still consuming less than one serving of WG per day (19–21). Reported barriers to WG consumption include inferior taste and texture in comparison to RG products, cost, availability, convenience, and lack of knowledge of the health benefits of WG (22, 23).

Research suggests that much of human behavior is driven not by conscious deliberation of immediate choices or concern over long term health outcomes, but rather by habit and other automatic processes that are extremely efficient (24–27). Numerous studies support this idea (28–30). Despite good dietary intentions, stress and increased cognitive load can impair an individual's ability to choose healthy options when presented with highly palatable, calorie-dense foods (31–33).

In addition to scenarios in which eating occurs as an automatic action triggered by powerful environmental or situational cues, other implicit processes may contribute to eating behavior and food choice. Social psychologists Greenwald and Banaji describe implicit attitudes as "introspectively unidentified…traces of past experience that mediate favorable or unfavorable feeling, thought, or action toward social objects" which "manifest as actions or judgments that are under the control of automatically activated evaluation" (34). Just as individuals make automatic, unconscious evaluations that determine judgments and actions in social situations, implicit attitudes toward particular foods may predict food choice. Studies investigating the influence of implicit attitudes on consumer food choice behavior have demonstrated that in some cases automatic associations may play a larger role than self-reported motivators such as perceived nutritional value or potential health benefits (35–38). A better understanding of implicit processes in human dietary choices may contribute to designing effective interventions to improve dietary behaviors such as consuming enough WG foods to achieve optimal health benefits.

Measures that capture implicit associations are widely used in consumer psychology, organizational management, and marketing research, as these fields recognize the limitations of self-reported explicit measures (35, 37, 39–41). One of the most widely used measures of implicit attitudes in nutrition research is the Implicit Association Test (IAT) (42–44). The IAT is designed to measure strengths of associations between contrasting target concepts and contrasting attributes by measuring response times in a computerized sorting task. The assumption underlying the interpretation of the IAT is that responses will be faster and more accurate when the target and attribute categories are more strongly associated. The measure is described as implicit because it operates without the test taker's awareness of the existence or strength of the associations under question.

The mere exposure paradigm consists of repeatedly exposing an individual to a novel or less liked stimulus object (45, 46). Although the individual is not required to engage in any kind of behavior or evaluation at the time of the exposure, it has been demonstrated that simply by mere exposure, acceptance and preference for the stimulus object can be enhanced (45–47). Research on acceptance of novel foods indicates that through repeated exposure, initially less palatable or unfamiliar foods ultimately achieve higher acceptance (48–51). If the mere exposure effect worked in our study, this would predict that people unfamiliar with WG would demonstrate increased liking and acceptability for these products after repeated exposures. Moreover, as implicit associations are rooted in experiences not consciously monitored or remembered, it is possible that mere exposure to WG foods in self-reported low WG consumers in this study could lead to a stronger implicit association between WG foods and pleasant taste.

The aims of the current study were to determine if: (1) initial implicit associations between WG/RG foods and taste (pleasant/unpleasant) predict consumption of provided WG products and (2) mere exposure to WG products incorporated into the daily diet of self-reported low WG consumers strengthens the association of WG foods with pleasant taste. If an implicit association between whole grain foods and pleasant taste can be strengthened by mere exposure, this may ultimately lead to consumers choosing whole grains.

2 Materials and methods

This pilot study was conducted in accordance with the ethical standards set by the University of California, Davis Office of Research Institutional Review Board (IRB ID 235561) and is registered at clinicaltrials.gov as NCT01403857. All participants provided written informed consent and received monetary compensation for their participation.

2.1 Participants

Healthy men and women, aged 20–45, with a body mass index between 18.5 and 32.0 kg/m^2 and stable body weight (within ± 3 kg) for the previous 6 months were recruited from Davis, California and outlying areas. Eligible participants prepared and ate the majority of their meals at home, and their habitual consumption of whole grains was ≤ 1 serving/day based on self-report. During the screening visit for the study, participants filled out an extensive questionnaire designed to assess the typical level of WG consumption (52). Questionnaire items included specific questions regarding the type, amount, and frequency of consumption of all grain products on a daily, weekly, biweekly, and monthly basis. Participants agreed to incorporate provided study foods into their daily diet for the duration of the 6-week intervention. Participants also agreed to continue their usual physical activity practices. Exclusion criteria included: currently dieting to lose weight; pregnant currently or within the past 6 months; diagnosis of type 1 or 2 diabetes; gastrointestinal diseases; regular use of colonics or laxatives; recent (within 3 months) use of antibiotics, appetite suppressants or mood-altering medications; regular use of tobacco products.

2.2 Study design

Details of this study have been previously described (52, 53). In brief, this pilot study was a 6-week parallel arm intervention study where participants were randomly assigned in permuted block sizes of 3 in a 2:1 ratio of those receiving WG to those receiving refined grain (RG) products (53). The reason for this ratio was that we were particularly interested in precisely measuring outcome variables in the WG group. The RG group functioned as a control group. The WG and the RG groups received the assigned grain products in weekly allotments containing the recommended number of grain servings based on individual caloric needs for weight maintenance. The provided grain products could be incorporated into meals or eaten as snacks throughout the day. Participants randomized to the WG intervention received WG products representing commonly consumed grain products in the US. Participants in the RG intervention received closely matching RG versions of the same foods. For the WG group, the products approached 100% of recommended total grain servings per day as WG; for the RG group, no WG products were provided. Provided grain product consumption was tracked by weekly logbooks used by participants.

To evaluate the effects of the intervention on implicit associations between WG and RG and taste, the Grains IAT was administered during a baseline test day prior to initiating the intervention and again on a second test day during the sixth week of intervention.

2.3 Grain products

A variety of grain products were provided by the USDA Western Human Nutrition Research Center (WHNRC) on a weekly basis to participants for 6 weeks. Some of the grain products were formulated and prepared by the Metabolic Kitchen and Human Feeding Lab in the WHNRC; others were commercially available foods (52, 53). Products were weighed and packaged without brand identification or nutrition information to avoid bias. Participants randomized to the WG group received WG sliced bread, ready-to-eat breakfast cereal, crackers, rice, couscous, penne pasta, spaghetti, tortillas, cookies, cornbread muffins, and baking mix. Some of the foods were provided both dry and cooked (couscous and pastas). The baking mix only required water to use for preparing pancakes or muffins. Participants in the RG group received closely matching RG versions of the same foods. Grain products were packaged and labeled according to the instructions for home storage food safety: room temperature (ready-to-eat), refrigerator, or freezer. The number of grain servings provided was based on the number of servings recommended for each participant's energy needs. For example, a participant with an estimated energy expenditure of 2000 calories per day would receive six servings of grains per day, for a total of 42 servings of grains per week. The estimation of resting energy expenditure for each participant was calculated using the Harris-Benedict equation (54), incorporating anthropometric data obtained during the screening visit. A light activity factor of 1.4 was used as a multiplier for resting energy expenditure to determine total daily energy expenditure.

2.4 Measuring exposure

In this study, mere exposure to WG was operationalized as provision of grain products during the 6-week intervention. Because all participants accepted into the study were self-reported low whole grain consumers, provision of WG to the WG group functioned as the mere exposure. Participants were instructed to record consumption of all grain products in a weekly log booklet, including grain products not included in study foods. Instruction was provided for recording accurate daily log entries, including details about preparation methods, amount, location, and time of day the grain products were consumed. Recipe suggestions, measurement aids, and blank notes pages were provided in the booklets. Participants returned unused and prepared-but-uneaten foods and all packaging materials along with the log booklet at the end of each week and then received the next week's allotment of products. Total servings of grain products consumed were calculated by measuring the disappearance of provided foods and by analyzing data recorded in the weekly logs. Participants were not required to consume all of the grain products provided each week but were encouraged to incorporate the provided foods into their daily meals and snacks in place of the products they would normally purchase for themselves. Participants were also instructed not to share their study foods with others.

2.5 Procedures

2.5.1 Test day protocol

Participants arrived on the morning of each test day after an overnight fast. After a short period for taking anthropometric and other study measures, they were given a standard light breakfast consisting of peach yogurt, apple slices, peanut butter, and bottled spring water. Approximately 45 min after completing breakfast, participants were escorted into a sound-proofed cognitive testing booth where they were seated in front of a desktop computer with a 17 in. flat screen monitor. The IAT task was administered using experiment generator software (Inquisit 3.0, Millisecond Software, Seattle, WA). Before starting the test, researchers read the on-screen instructions aloud and confirmed participants' understanding of the task procedures. After starting up the test, the investigator exited the cognitive testing booth but remained in the general area in the case a participant had any questions or concerns during the testing. Participants were told that the purpose of the computer test was to gather information about their food preferences. Thus, they were unaware that their reaction speed was being measured.

2.5.2 The implicit association test

The IAT Is a computerized test that indirectly measures the strength of association between pairs of concepts: (a) two contrasted target categories and (b) two contrasted attribute categories via a classification task. Stimuli for the four categories (Supplementary Table S1) in this study included: (1) photographic images of RG foods, (2) photographic images of WG foods, (3) words associated with tastiness or enjoyment of food (e.g., tasty, delicious), and (4) words associated with lack of taste or enjoyment (e.g., flavorless, unappealing). Participants were instructed to rapidly classify stimuli that represented target and attribute into one of four distinct categories as quickly and accurately as possible using only two response keys on the computer keyboard. The food image or taste word appeared in the center of the computer screen and stayed onscreen until the participant responded. In the case of an incorrect response (e.g., pressing the key for "Refined grain" for an image of a whole grain food), a red "X" appeared on the screen and the participant had to correct the response in order to continue. An inter-stimulus interval of 250 ms was used. Response latency was measured in milliseconds, providing a measure of the strength of association between target and attribute.

The IAT started with practice blocks in which only images of WG or RG foods or words relating to positive or negative taste appeared on the screen and participants classified them using the E or I key on the keyboard. Participants referred to descriptors positioned on the top left and right corners of the computer screen which indicated the correct response (Supplementary Figure S1). These descriptors remained on the screen for the duration of each practice and test block. The task became more complex in subsequent practice and test blocks where the categories were combined, and the participants had to sort a WG or RG grain food with a positive or negative taste word using only two keys. In the combined practice and test blocks, participants were instructed to use the same key for either WG or RG *and* positive or negative taste. The idea being that the stronger the implicit association between target and attribute, the faster the response time would be. In the second set of combined blocks the target-attribute combination was reversed. Table 1 lists the order of practice and test blocks, which is consistent with that described by the originators of the IAT (55, 56). For each participant session, the order of stimulus presentation was randomized within each practice and test block.

Response time measured in milliseconds was used to calculate an IAT *D* score averaged over all participants and for each individual participant, as recommended by Greenwald et al. (56). The *D* score is computed as the difference in average response time between the IAT's two combined tasks (e.g., RG and good taste, WG and bad taste; WG and good taste, RG and bad taste), divided by a pooled standard deviation of participant response times in the two combined tasks (Table 2). The resultant statistic is an effect size similar to a Cohen's *d* effect size, the main difference being that the standard deviation in the denominator of *D* is calculated from the scores in both conditions, whereas the Cohen's *d* score is computed using a pooled within-treatment standard deviation (56). Using this algorithm, the resultant measure is the IAT *D* effect. This *D* score indicates overall implicit association between RG and WG foods and good or bad taste. We designed the computer program so that positive values of this score indicated faster reaction times in the 'congruent' blocks in which RG foods and pleasant taste shared the same response key, signifying a stronger implicit association between RG foods and pleasant taste, as compared to WG foods and pleasant taste; negative values indicated the reverse associations. Presentation order of the congruent and incongruent test blocks was counterbalanced among participants. A score of zero indicates no difference in preference for WG or RG foods.

3 Statistical analysis

Data were exported from Inquisit to Microsoft Excel for preparation and analyzed using SAS for Windows Release 9.4 (Cary, NC, United States). The number of weekly servings of grain products provided to participants varied according to caloric needs for weight maintenance, thus percentage of provided products consumed was used in the analysis to compare the difference in consumption between groups. Grain product consumption and *D* scores were assessed for conformance to the normal distribution using the Shapiro–Wilk test. None of the variables needed to be transformed as they were all normally distributed. We tested for outliers in the data, identified as >3 SD from the mean, but none were found. Baseline *D* score was included in models as a continuous variable. Participant characteristics at baseline were compared between groups with Wilcoxon tests and chi-square tests. Percentage of provided products consumed at week 1, percentage of products consumed averaged

TABLE 1 Sequence of trial blocks used in the implicit attitude test[1].

Block	Number of trials	Function	Items assigned to left key	Items assigned to right key
1	20	Practice	RG food	WG food
2	28	Practice	Positive taste	Negative taste
3	24	Practice	RG and positive taste	WG and negative taste
4	48	**Test**	**RG and positive taste**	**WG and negative taste**
5	40	Practice	Negative taste	Positive taste
6	24	Practice	RG and negative taste	WG and positive taste
7	48	**Test**	**RG and negative taste**	**WG and positive taste**

[1]RG, refined grain; WG, whole grain. The bolding is for emphasis of the differences and adds meaning to the understanding of the tests.

TABLE 2 Summary of IAT scoring procedures recommended by Greenwald et al. (56).

1. Delete all trials greater than 10,000 milliseconds.
2. Exclude participants whose response times were less than 300 milliseconds on more than 10% of trials.
3. Compute one pooled standard deviation for all trials in Blocks 3 and 6; another for all trials in Blocks 4 and 7.
4. Compute the mean of correct latencies for each of Blocks 3, 4, 6, and 7.
5. Compute the two mean differences (Mean Block 6 – Mean Block 3) and (Mean Block 7 – Mean Block 4).
6. Divide each difference score by its associated pooled-trials standard deviation from step 3.
7. *D* score is the equal-weight average of the two resulting ratios in step 6.

across all 6 weeks, and change in IAT *D*-scores were compared between groups with analysis of covariance (ANCOVA), controlling for initial *D* score or grain type (WG vs. RG) preference, age, and sex of the participant. Change in percentage of products consumed from week 1 to week 6 was compared between groups with two-sample *t*-test. Tests were two-sided and significance level was set at $p < 0.05$. Data are presented as means ± SEMs unless otherwise noted.

4 Results

4.1 Recruitment

The CONSORT flowchart of participants in this study is shown in Figure 1. Of the 63 participants who were enrolled, eight withdrew before randomization, leaving 55 participants to be randomized into either the WG or the RG intervention. Nine participants dropped out of the WG group for a variety of personal reasons; however, none stated dissatisfaction with study foods as the reason. One person in each group was lost to follow-up, no reason given, leaving 45 participants to complete the intervention. Although the randomization scheme was intended to result in a 2:1 ratio of WG to RG group sizes, the final number of completers totaled 34 in the WG group and 11 in the RG group, due in part to early termination of the study for funding reasons. Although the total number of participants was fewer than planned, the number of participants in the WG group exceeded the minimum required in our sample size calculation to be powered to measure changes in *D* scores, which was 30 participants for the WG group.

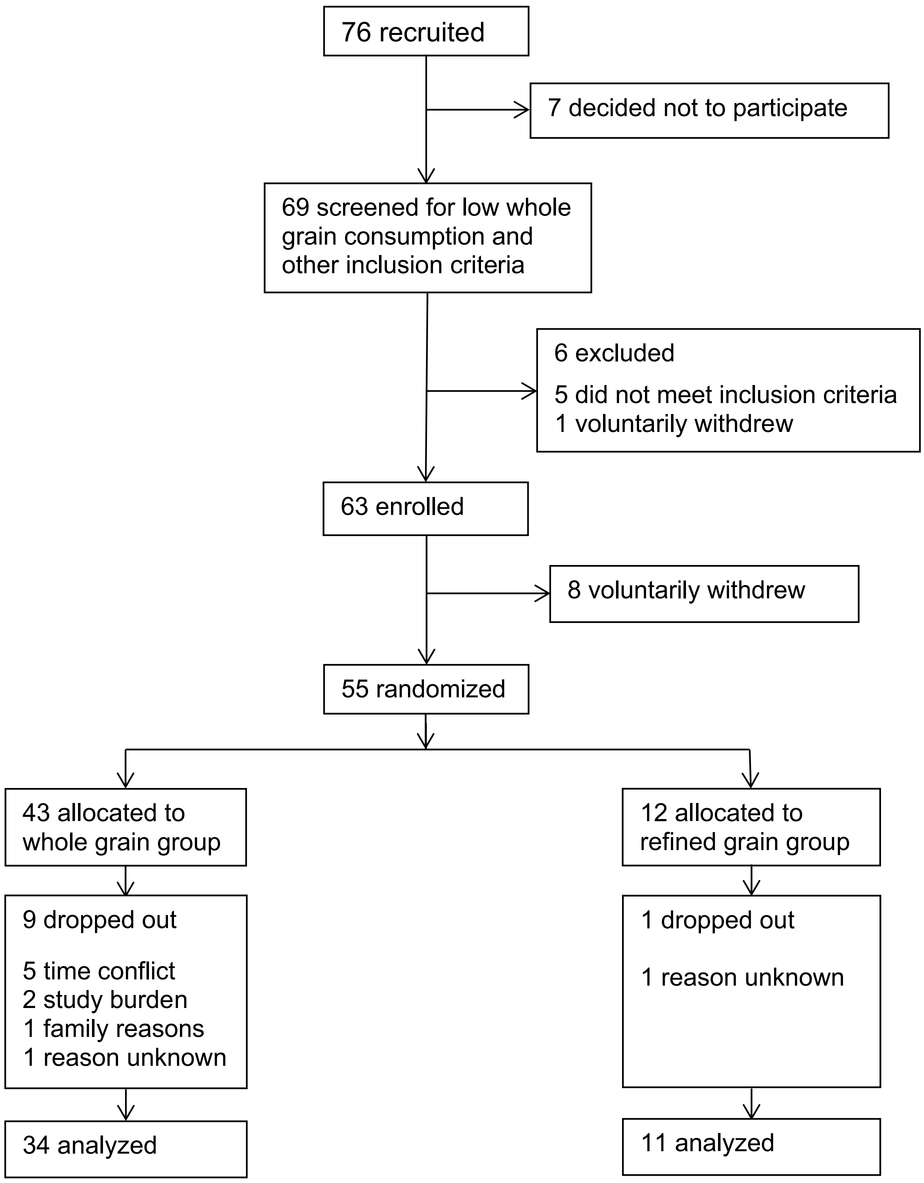

FIGURE 1
CONSORT flowchart of participants through the study.

TABLE 3 Participant characteristics.

	Whole grain (*n* = 32)	Refined grain (*n* = 11)
Age (*y*)[2]	25.4 ± 6.0	24.2 ± 5.5
Sex (n)		
Male	12	8
Female	20	3
BMI (kg/m^2)[2]	22.6 ± 2.6	25.6 ± 6.6
Initial *D* scores[3]	−0.175 ± 0.588	−0.156 ± 0.644
Prefer WG	21	7
Prefer RG	11	4

[1]BMI, body mass index; WG, whole grain; RG, refined grain.
[2]Values are means ± standard deviations.
[3]A negative D score indicates a preference for WG and a positive D score indicates a preference for RG.

4.2 Participant characteristics

Of the 45 participants who completed the study, results from 43 are presented here. Technical difficulties prevented two participants from completing the computer IAT at the baseline visit. No significant differences were found between the whole grain and the refined grain group regarding age ($p = 0.31$), BMI ($p = 0.35$), or initial D score ($p = 0.96$), but there were significantly more female participants in the WG group than in the RG group ($p = 0.043$) (Table 3).

Despite being self-reported low whole grains consumers, the majority of participants (28 out of 43, or 65%) had baseline IAT *D* scores indicating an implicit preference for whole grain foods over refined grain foods. This preference was well distributed between treatment groups. Participants who received the WG products consumed an average of 48 ± 3% of the foods provided over the 6-week intervention period, and those receiving the RG products consumed 45 ± 8% over the same time period.

4.3 Implicit taste preference and grain product consumption

The primary aim of this study was to determine if initial implicit taste preference for WG or RG would predict consumption of provided grain products. Thus, we examined the relationship between the initial *D* score and the percentage of provided grain products consumed during the first week of the study and again for all 6 weeks of the study. Within each treatment group, there was no association between consumption at week 1 and initial *D* score, WG foods ($r = 0.242$, $p = 0.462$) and RG foods ($r = 0.012$, $p = 0.796$). Combining the results of both treatment groups, there was also no association between consumption at week 1 and initial *D* score ($r = 0.246$, $p = 0.990$). Similarly, we found no association between initial *D* score and percentage of grain products consumed over the full 6-week period within each treatment group, WG foods ($r = 0.197$, $p = 0.626$) and RG foods ($r = 0.424$, $p = 0.311$), respectively, or when combining results of both treatment groups ($r = 0.285$, $p = 0.162$) (Figure 2).

Consumption of provided grain products did not change over time in participants who received the WG products. Further, we found that consumption of the WG products did not change from beginning to end of the intervention regardless of whether the participant preferred whole or refined grains initially (Table 4). Considering that participants in the WG consumed nearly half of the provided grain foods, this represents a significant increase in WG consumption in individuals who self-identified as consuming ≤1 serving of WG/day. However, for the participants who were assigned the RG foods, those who showed an initial preference for RG foods consumed less of the provided grain products at week 6 than at week 1 ($p = 0.041$) (Table 4).

4.4 Effect of exposure on implicit associations

Another aim of this study was to determine if by simply providing WG foods to low WG consumers, thereby creating a mere exposure effect, implicit attitudes toward WG could be changed. The IAT *D* scores at baseline and week 6 were summarized for both groups to determine if exposure to the whole or refined grains changed the strength of association of WG foods with good taste. Controlling for initial *D* score, the change in *D* score was different between the WG and the RG intervention groups, ($p = 0.034$). There was no change in *D* score at 6 weeks in the WG intervention group, whereas the RG intervention group had a significant decrease in the *D* score, indicating a preference for whole grains (toward WG preference) (Figure 3).

For participants who initially preferred WG, this preference did not change regardless of whether they received the RG or the WG products (Figure 4). For participants who initially preferred RG, there was a significant shift away from preferring RG and toward preferring WG at week 6. This shift was apparent in the group receiving RG foods ($p = 0.004$) and also occurred in the group receiving WG foods ($p = 0.032$) (Figure 5).

5 Discussion

To our knowledge, this is the first study to utilize the Implicit Association Test as both a predictor and an outcome of mere exposure to a prolonged dietary intervention in a free-living setting. The Implicit Association Test measures the strength of association between a target concept and attribute; an implicit association is a non-conscious result of past experience which has been internalized and is not available to conscious introspection. Implicit measures may have better predictive validity regarding food choice than explicit measures, especially in situations where cognitive or emotional

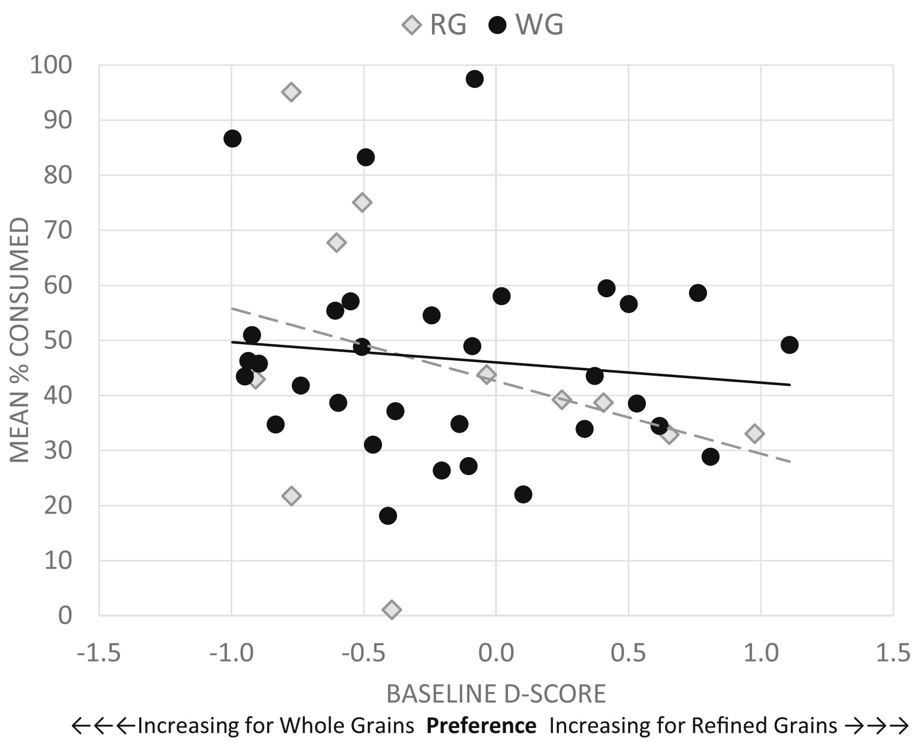

FIGURE 2
Percentage consumption averaged over the 6-week intervention for the whole grain (WG) and refined grain (RG) groups. Neither the WG group nor the RG group showed an association between the baseline D score and the percent of grain products consumed over the 6-week intervention.

TABLE 4 Consumption of grain products by participants according to initial preference[1].

	Whole grain group		Refined grain group	
Initial implicit Preference	WG $n=21$	RG $n=11$	WG $n=7$	RG $n=4$
% of Grain products consumed[1]				
Week 1	49.2 ± 4.2	51.9 ± 4.0	43.3 ± 11.2	45.5 ± 5.0
Week 6	43.0 ± 5.0	40.8 ± 8.1	56.2 ± 13.2	31.7 ± 2.5
Change in % Wk1 – Wk6	−6.2 ± 4.5	−11.1 ± 10.0	13.0 ± 7.7	**−13.8 ± 4.0***

The bolding is for emphasis of the differences and adds meaning to the understanding of the tests.

resources are limited due to stress or other factors (28–30). In this study, we aimed to see if an implicit association between refined or whole grain foods and taste would predict consumption of provided WG foods in low WG consumers in a free-living situation. We questioned if by merely exposing participants to WG, thereby creating new experience, would participants' implicit association between those foods and taste be altered. Specifically, would mere exposure to WG result in a more positive *implicit* association between WG and taste? Furthermore, if the implicit association between WG and good taste was strengthened, would this result in increased consumption of those foods?

Our results were unexpected and intriguing. First of all, despite being self-identified low WG consumers, the majority of participants had a stronger implicit association of WG foods with pleasant taste than RG foods with pleasant taste before the intervention, based on their baseline IAT test scores. At no point during the screening process for the study did we ask participants whether they preferred refined or whole grain foods. Prospective participants were simply informed that this was a study "evaluating liking, acceptability, and health benefits of grain products." The words "whole grain" and "refined grain" were never spoken to participants, and as they entered the study and received their study foods individually, participants were not aware that some received different products than they did. We did not want to bias their consumption of provided products by drawing attention to the perceived healthiness of the foods. Studies by Raghunathan et al. (38) demonstrated that for some individuals, foods perceived as 'healthy' are automatically associated with not being tasty, and this could have negatively affected consumption of provided study WG foods in the current study. Although we did not employ an explicit measure of preference for whole or refined grain foods, we thought it likely that low WG consumption would at least in part be due to a greater preference for the taste of RG foods over WG foods. Despite research indicating

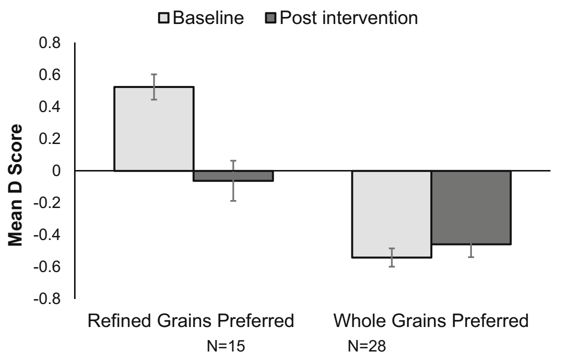

FIGURE 3
Implicit Preference for Grain Foods as Measured by D Score for all Participants. Bars represent mean IAT D scores with standard error bars. An implicit preference for refined grains is indicated by positive scores. At baseline, 15 of the 43 subjects preferred refined grains (left bars); at the end of intervention this preference changed (p<0.001) such that there was no clear preference for refined or whole grains. An implicit preference for whole grains is indicated by negative scores. At baseline, 28 of the 43 subjects preferred whole grains (right bars); this preference did not change at the end of intervention.

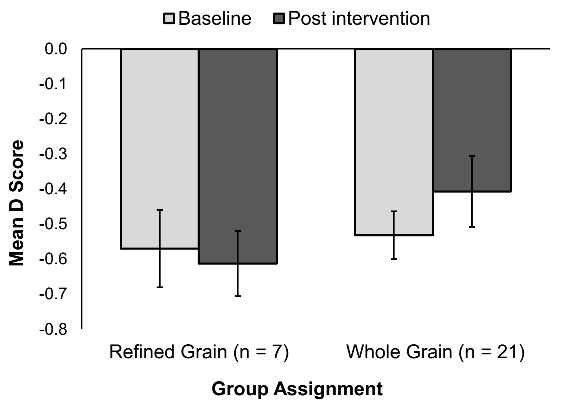

FIGURE 4
D scores in the 28 participants initially preferring whole grains, illustrated for groups receiving either refined grain products (left set of bars) or whole grain products (right set of bars). Bars represent mean IAT D scores with standard error bars. An implicit preference for whole grains is indicated by negative scores—responding more quickly in trials when whole grain food images were paired with positive taste descriptors and refined grain food images were paired with negative taste descriptors.

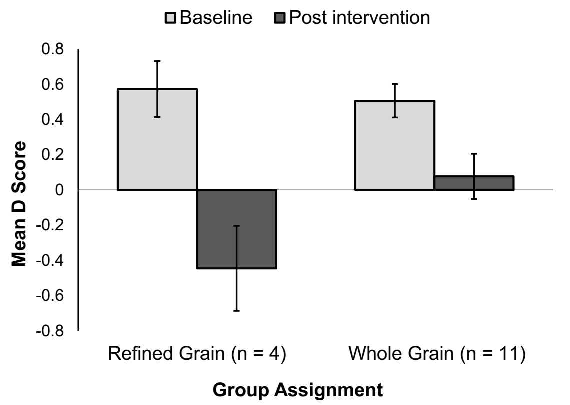

FIGURE 5
D scores in the 15 participants initially preferring refined grains. Bars represent mean IAT D scores with standard error bars. Positive scores indicate an implicit preference for refined grains and negatives scores indicate an implicit preference for whole grains.

that the bitter taste of WG foods can negatively influence acceptability and consumption of WG products (57), the results of our initial Implicit Association Test suggest otherwise.

In answer to the first question, to determine if initial implicit associations between WG foods and taste would predict consumption of provided WG products, the answer was that it did not. Our results showed no difference in consumption of provided WG foods based on initial preference for whole or refined grains, as based on baseline participant IAT scores. In other words, even individuals with a more positive initial implicit attitude toward RG consumed the provided WG foods. These results are not entirely surprising given that the literature on the ability of the IAT to predict behavioral food choices has been mixed. Perugini (36) compared the predictive validity of implicit attitudes with that of explicit attitudes toward fruits versus snack foods and found that implicit attitudes, as measured by an IAT, better predicted a behavioral choice between a free piece of fruit or a snack (36). In contrast, Karpinski and Hilton (58) found that while both the IAT and explicit attitudes toward apples and candy bars showed the same preference, only the explicit attitude predicted behavioral choice. A number of different explanations for these discrepancies has been suggested. Ayres et al. (59) expanded on previous research and found that perceived palatability of food may influence the prediction of food choice beyond implicit measures. Meissner et al. (60) further explained how extraneous influences, such as task recoding, can affect the validity of the IAT and other implicit measures in predicting behavioral outcomes. That being taken into consideration, our results showing consumption of WG products in self-reported low WG consumers in a free-living setting suggest that switching RG for WG products is behaviorally feasible with little thought or effort.

In this study, mere exposure to WG in self-reported low WG consumers did not lead to a greater implicit association of WG with good taste or an increase in WG consumption over the course of the study. It is possible that the ability to change the implicit preference for WG or to increase WG consumption was hampered by the fact that the majority of participants implicitly associated WG with good taste at the start of the study. As the initial *D* scores were already indicative of a positive association, there may have remained little room for a measurable change in the positive direction (i.e., a ceiling effect). Future research with a longer-term exposure period to WG and RG foods may provide different outcomes with a more measurable change in preference and consumption of WG by individuals who self-identify as low WG consumers.

In participants who initially implicitly preferred RG foods and who were assigned the RG products, consumption of those foods declined over the 6-week intervention, whereas consumption of provided RG foods did not change over time in those who indicated a preference for WG foods initially. Considering all participants, the implicit association of RG foods with good taste declined between baseline and week 6. This shift occurred both in participants assigned

RG and WG, although the shift was greater in individuals given RG products.

Our results showing decreased consumption of RG foods support the findings of Reynor and colleagues (61) who reported a 'monotony effect', which is defined as different from 'sensory specific satiety' (62). Sensory specific satiety refers to a phenomenon occurring within or shortly after a meal, tending to be of short duration, and the monotony effect refers to the decrease in perceived pleasantness of foods resulting from tasting the same flavors over time. The monotony effect tends to be of longer duration than that of sensory specific satiety (62). In their experiments Raynor and colleagues (63) found that by reducing dietary variety, consumption of provided food groups decreased. In the present study, it is possible that by providing self-reported RG consumers with an abundance of RG foods, we stimulated the monotony effect which resulted in decreased consumption of provided RG food and a greater implicit association of WG with pleasant taste. Zandstra et al. (64) found that when participants were allowed the greatest variety of choice in a study of product acceptance and in-home consumption of a meat sauce consumed once a week for 10 weeks, boredom ratings were lowest and acceptance rating highest in the group that was afforded the greatest variety. Given that the participants in the RG intervention group were presumably already consuming RG versions of provided study foods, this might explain the greater implicit association of images of WG grain foods and pleasant taste in the IAT task. However, given the small size of the group receiving the RG products, these findings should be considered with caution. In addition to reduced likelihood of detecting true effects, small sample sizes may conversely increase the odds of statistically significant results that are actually spurious.

It should be noted that the consumption of provided grain products averaged over the 6-week period was not different between intervention groups, with both groups consuming slightly less than half of grains servings provided. While participants were not restricted to consuming only the grain foods provided by the study, they were instructed to log all grain products in their weekly log booklets. The analyses we conducted were based only on the study foods because we could measure disappearance and check it against the log booklets. It would be informative to follow up this study with one where the non-study foods could also be included in the analyses, but this would require a method to accurately log outside foods. Advances in technology, such as smart phones with cameras to snap photographs of meals, may present an avenue for better documentation of food consumption in free living situations, not only for WG intake, but for all foods.

Whereas the present analysis is concerned with implicit processes that may be influenced by exposure to WG and concomitant effects on consumption of provided WG products, we have previously reported on health-related parameters associated with increased WG consumption in low WG consumers (52). We have also reported on the results of standard sensory evaluation testing of whole and refined grain foods before and after a 6-week exposure period to either WG or RG (53). We aimed to ascertain the specific sensory attributes of WG foods (e.g., overall liking, appearance, flavor, texture) that contribute to the willingness to include them in the regular diet. In addition, we investigated changes in implicit and explicit liking and wanting for other foods varying in fat content (high/low) and taste (sweet/savory) as result of exposure to WG as a potential health halo effect (53). Taken together, our published results contribute to understanding factors that lead to acceptance and liking of WG foods in addition to measuring clinical and physiological changes resulting from increased WG consumption.

Strengths of this study include the use of an implicit measure, the IAT, to investigate the influence of automatic attitudes on WG consumption in a free-living situation. Behavioral outcomes of nutrition interventions utilizing implicit measures in laboratory settings may not be generalizable outside of those settings. Additionally, using both disappearance and recorded consumption data allowed us to ascertain the effect of mere exposure to WG foods on implicit attitudes in healthy adults who were self-reported low WG consumers. By supplying participants with WG products in above the recommended amounts, we eliminated some of the commonly cited barriers to WG consumption, including cost, availability, and ability to identify WG at the point of purchase. Although poor taste has been reported to be the largest barrier to WG consumption, results from this study suggest that other barriers, such as texture, appearance, or cultural factors may play a more important role. This research contributes to the existing knowledge regarding barriers and facilitators to WG consumption and suggests further questions to investigate for increasing consumption of this important food group.

Limitations of this study include the inability to directly observe consumption of provided grain products. Participants were instructed to record intake of grain foods in provided log booklets, leaving data collection vulnerable to self-report bias. Although the free-living nature of the intervention was intended to deliver a more realistic picture of grain consumption, this entailed the inability to control for sharing of provided foods with others, simply discarding provided foods, or the addition of other grain products from outside sources. Future studies could be improved by including a validated physiological biomarker of whole grain consumption, such as plasma or urinary alkyl resorcinols to separate results from compliers and non-compliers in the outcomes of interest (65–67). A serious limitation to the present study was the overall small sample size and the unevenness of group sizes, which limited our power to detect group differences. Furthermore, in studies aimed at assessing barriers to WG consumption using implicit measures, the addition of an explicit measure of preference for one grain type over the other could provide information about which type of measure, implicit or explicit, better predicts WG consumption in a free-living situation. Previous studies have compared predictive validity of implicit and explicit measures in a variety of laboratory settings, which may not be indicative of behavior in real world settings (39, 42, 68, 69).

6 Conclusion

In this study, we aimed to determine the effects of implicit associations between whole and refined grains and taste and the effect of mere exposure on implicit associations and on consumption of provided grain foods. Initial preference for WG or RG foods did not predict consumption of WG products; consumption of provided WG foods did not differ between those who initially preferred refined or whole grains. Interestingly, we found that individuals who initially preferred RG foods decreased consumption of provided RG foods but not of provided WG foods. We questioned whether by mere exposure, implicit associations between WG foods and good taste would increase, which did happen, but only in the group initially preferring RG. On the other hand, implicit preference for RG decreased after 6 weeks of exposure to both refined and whole grains groups. This suggests that mechanisms other than initial taste preference may be at the root of choosing RG over WG. Future research into ways to replace some RG with WG, rather than focusing solely on increasing WG consumption, may represent an alternative strategy to support the Dietary Guidelines for Americans recommendations for grain intake to achieve health benefits.

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 University of North Dakota Institutional Review Board. 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

AL: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. DB: Conceptualization, Investigation, Methodology, Resources, Writing – review & editing. BR: Conceptualization, Investigation, Methodology, Writing – review & editing. ML: Conceptualization, Methodology, Resources, Software, Writing – review & editing. NK: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

ML was employed by the VTT Technical Research Centre of Finland Ltd.

The remaining 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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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1408256/full#supplementary-material

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