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CHINESE NUTRITION SOCIETY

The **Chinese Nutrition Society (CNS)** is a non-profit professional organization dedicated to bring academics, education, research institutions, and industries together to advance research and application of nutrition science for the promotion of human well-being and disease prevention. CNS acts as a platform in the development of nutrition science in China.

CNS was founded in 1945 and was officially re-established by the Chinese Association for Physiological Sciences (CAST) later in 1985. CNS joined the International Union of Nutrition Science (IUNS) and became a member of the Federation of Asian Nutrition Societies (FANS) in 1984 and 1985 respectively. CNS has a continuously growing number of over 20,000 members from 31 regional societies across China; they include academics, nutritionists, clinicians and dietitians, health workers, educators and students. The headquarters of CNS is in Beijing. Prof Yuexin Yang, the 6th and current president, has led CNS since 2013.

The main activities of CNS include the hosting of the Chinese Nutrition Science Conference (CNSC) which is held biannually. CNS is also mainly responsible for the National Nutrition Week in China which advocates the importance of nutrition. Both the Chinese Dietary Guidelines (CDG) and Chinese Dietary Reference Intakes (Chinese DRIs) are established and revised by CNS. In addition, CNS is responsible for the training and continuing education of China's registered dietitians. It is also involved in events involving the exchange, promotion and dissemination of nutritional knowledge.

The main missions of CNS are: (1) to bring researchers and scientific workers together to improve science and education; (2) to develop and extend nutrition science and technology in food industry and dietary practice; (3) to support the dissemination and application of nutrition science to improve public health and clinical practice and; (4) to promote education and training of dietitians, and advocate the development and implementation of food and nutrition policies.

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Asia Pacific Clinical Nutrition Society Award for 2022

Professor Shaw Watanabe MD, PhD

Immediate past president, Asia Pacific Clinical Nutrition Society

Shaw Watanabe was born in Pyongyang on January 18th 1941 and graduated from Keio University School of Medicine in 1965. After completing his internship, he entered Keio Graduate School and majored in human pathology. As a distinguished haematopathologist at the National Cancer Institute (U.S.A.) and the Pathology Division, National Cancer Center (Japan), he defined criteria for T-cell lymphoma/leukemia and Letterer Siwe disease by defining dendritic cells with the S-100 protein. He was then appointed Director of the Epidemiology Division, National Cancer Center (1985–1996), where he established a population-based cohort study (JPHC), in which 140,000 residents in eleven health center districts participated. As a consultant, he also contributed to the success of the WHO Framework Convention on Tobacco Control and the IARC Fellowship Selection Committee member.

After being diagnosed with diabetes at the age of fifty, he moved to Tokyo University of Agriculture (1995–2005) to promote diabetic control through appropriate diet and physical activity. As a professor of public nutrition, he became one of the pioneers of the use of functional foods. He was promoted Director General, National Institute of Health and Nutrition (2005–2009), and commissioned by the Cabinet Office to develop a national policy on Shoku-iku (Eating education). After retirement he became the President of the Life Science Promoting Association (2009–2020) and the President of the Japanese Society of Integrative Medicine (2011–2020). He chaired the Asia Pacific Conference on Clinical Nutrition 2014 in Tokyo and the East Asia Conference on Standardization of Rice Function in Kyoto.

At the age of eighty, he moved to the Life Science Promotion Association at the Louis Pasteur Medical Research Institute in Kyoto He has concentrated on the research and popularization of brown rice, and establishing the Medical Rice Association aimed at "health promotion with brown rice." He has registered the platform, a “Place for Accumulation and Utilization of Knowledge" with the Ministry of Agriculture, Forestry and Fisheries and launched a consortium for the use of processed low-protein brown rice for kidney disease patients and organic brown rice for’ healthy longevity.’

He was Editor of the Journal of Epidemiology from1996 to 2005 and is currently an associate editor of the ‘Asia Pacific Journal of Clinical Nutrition’ with editorial roles for ‘Clinical & Functional Nutriology’, ‘Life Science’, ‘Diabetes Research Open’ and ‘Nutrients’. He is an emeritus member of the Japan Pathological Society, the Japan Epidemiology Society, the Society of Functional Food Factors, and other academic societies. He has been a member of scientific boards of the Ministry of Health, Labour and Welfare; the Ministry of Agriculture, Forestry and Fishery; and the Cabinet Office and Environmental Agency in Japan. Internationally, he has worked with WHO (Tobacco control) and IARC (the International Agency for Research on Cancer Dioxin report).

He has been recognised with the WHO Tobacco or Health Medal (1991), the Japan Medical Association Award (1993), the Japan Epidemiological Association Award (2000), and the Award for Contribution to Society from Keio University Medical School (2014).

He is currently the President of the Life Science Promotion Foundation in Japan and was President of the Asia Pacific Clinical Nutrition Society from 2018 to 2021

His academic career has been distinguished by notable appointments:

- Lecturer, Department of Pathology, Keio University School of Medicine (1970-73)
- Assistant Professor, Department of Pathology, Keio University School of Medicine (1973-77)
- Visiting Scientist, Pathology Division, National Cancer Institute, USA (1975-76)
- Section Head, Pathology Division, National Cancer Center Research Institute, USA (1977-1985)
- Chief, Epidemiology Division, National Cancer Center Research Institute (1985-1996)
- Director, Tobacco or Health Research Collaborating Center (1985-1995)
- Professor of Pathology, Keio University School of Medicine (1985-present)
- Professor and Chairman, Department of Nutrition and Epidemiology, Tokyo University of Agriculture (1995-2005)
- Director General, National Institute of Health and Nutrition (2005-2009)
- President, Life Science Promotion Foundation (2009- 2020)
- President, Asia Pacific Clinical Nutrition Society (2018-2021)

and further responsibilities as:

- Board member, Shokuiku Promotion Committee, Cabinet Office, Japan (2005-2010)
- Chemical Substance Regulation Law (2006-2007)
- Board member of Health and Science Council, Ministry of Health, Labour and Welfare (2005- 2009)

- Board member, Japan Anti-Aging Medicine (2005-present)
- Board member, Japanese Society of Clinical Nutrition (2004-2010)
- Board member, Japan Antioxidant Unit Study Group (2005-present)

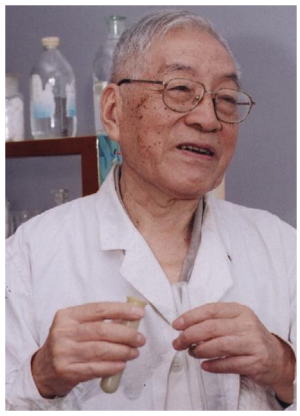
The Asia Pacific Clinical Nutrition Society Award for 2022 is made to Shaw Watanabe in recognition of his contributions to the nutritional well-being of peoples in the Asia Pacific region.

Citation by

Professor Duo Li MD, PhD

Chair of Nomination Committee
Asia Pacific Clinical Nutrition Society Award

Obituary for Professor Jingfan Gu (1927-2022)



Professor Jingfan Gu, an outstanding nutritionist, died on February 22, 2022, at the age of 95. The death of Professor Jingfan Gu deeply grieves us! His death is a great loss in nutrition circles.

Gu Jingfan was born in Wuxi, China in 1927. He graduated from Yanjing University in 1948 and Peking Union Medical College in 1952. He became the director of the ***Institute of Hygiene and Environmental Medicine of the Academy of Military Medical Sciences (based in Tianjin)***, the director of the Military Nutrition and Food Hygiene Inspection and Research Center, a member of the Medical Science and Technology Committee of the Ministry of Health of the General Logistics Department, and leader of the Military Health Professional Group.

His contributions to ***nutrition science institutions*** at home and abroad were impressive. Professor Gu was a founder of the re-established ***Chinese Nutrition Society***. From 1979, he served, in turn, as a member of the preparatory group, secretary general, vice president and president of the Chinese Nutrition Society. In 1985, he led the delegation which successfully bid for membership of the ***International Union of Nutritional Sciences (IUNS)*** at its Brighton, UK, conference, and played a role in its Expert Committees. He was Executive Director and Secretary General of the 7th ***Asian Federation of Nutritional Societies (FANS***). Professor Gu was a founder of the ***Tianjin Nutrition Society***, for which he successively served as chair of its first and second councils and honorary chair of its third Council.

Despite his age and extensive responsibilities, Professor Gu always maintained his enthusiasm and investment in the activities of the ***Chinese Nutrition Society***, with silent and dedicated effort and wisdom, making outstanding contributions to the development and growth of nutrition science and policy in China. He received the ***Asia Pacific Society of Clinical Nutrition Award*** for 1995; and in 2005 rated an ***"Internationally Renowned Scholar"*** at the 18th ICN-IUNS in South Africa; He was bestowed the honorary title as one of "A Generation of Famous Science and Technology Teachers" of the General Logistics Department of the PLA; the Outstanding Achievement Award of the China Nutrition Society in 2010 and the Lifetime Contribution Award of the Chinese Nutrition Society in 2010; and named "National Outstanding Scientific and Technological Worker" of the Chinese Association for Science and Technology.

Professor Gu always attached great importance to both ***basic and applied nutrition research***, which prospered nutrition evaluation, nutritional requirements, prevention and treatment of nutritional deficiency diseases and ***nutritional support for war trauma***. During 1956 to1959, he evaluated nutrient requirements of the infantry and cavalry in Tonghua and Sanshenggong (now Dengkou County, Inner Mongolia Autonomous Region, China) of relevance to residents and soldiers. In 1974, he introduced the glutathione reducing activity coefficient method to evaluate riboflavin status with minimal whole blood in China. In 1979, he pursued the need to address ***metabolic changes after burns***. For this, he was awarded the first Academic Prize of the Hou Xiangchuan Foundation of the Chinese Nutrition Society in 1988. In 1978 and 1981, he developed a general-purpose and trauma-specific crystalline amino acid intravenous injection, the first ***parenteral nutrition*** preparation in China. The study was recognised by prizes in Military Scientific and Technological Achievement and Scientific and Technological Progress. He studied ***nutritional metabolism in tropical environments***, focusing on the role of vitamins in improving heat resistance, developing, for example, long-acting vitamin B1 and B2 oil suspension injections, and heat-resistant vitamin C derivatives. He compiled and published the first Atlas of ***Wild Vegetables in China***.

From 1981, with the Institute of Hygiene and Environmental Medicine of the Academy of Military Medical Sciences, Professor Gu resumed publication of ***Acta Nutrimenta Sinica***. He served, in turn, its associate editor, editor-in-chief and honorary editor-in-chief. Professor Gu, together with the Chinese Nutrition Society, revised the "***Chinese Dietary Reference Intakes***" and "***Dietary Guidelines for Chinese Residents"***, and published a ***History of Chinese Nutrition*** and Biography of the Previous Generation of Nutritionists.

Even beyond the age of 80, he reviewed manuscripts and often worked late into the night. He was careful and meticulous in his reviews, and helpful to authors. His ***academic rigour*** has left a deep impression on many authors.

Under his leadership, ***Acta Nutrimenta Sinica*** won national journal awards, and was rated among "One Hundred Chinese Outstanding Academic Journals" and considered a "Chinese High-Quality Scientific and Technological Journal".

Professor Gu published more than 180 papers both in China and abroad. In 1990, he published ***Clinical Nutrition*** with Professor Hou Xiangchuan, based on the WHO-IUNS Manual developed by Wahlqvist and Vobecky, and jointly written with nutritionists and physicians in China. It triggered the wide development of Clinical Nutrition across China, and became an important guide to nutrition practice. He edited 11 monographs, including Intravenous Nutrition, Enteral Nutrition, Modern Clinical Nutrition, and a Special Nutrition and Nutritional Recipe Series. He was a co-founder of the

Asia Pacific Journal of Clinical Nutrition in 1992, encouraging its provision of Abstracts in Chinese during the first 2 decades of its history, and its presence in Chinese University hospital libraries nationwide.

Professor Gu displayed rigorous scholarship, modesty, simplicity, and approachability. He was kind and patient with his younger peers, for whom he gave careful guidance, allowing opportunity for discussion of ideas, hypotheses, study design, interpretation, ethical considerations, written reports, and communication. Being busy was no barrier. He was an exemplary role model, as leader, teacher, researcher, practitioner, and link to a wider world of nutrition science and scientists. He played a pivotal role in the re-emergence of nutrition in public health, clinical practice, and policy in China and beyond. His inspiring spirit, intelligence, wisdom, professionalism, supportive personality and longevity allowed his dedication to nutrition science to be a transformative factor in China's journey towards better health and wellbeing.

Citation by

The Editors
Asia Pacific Journal of Clinical Nutrition

10th China Nutrition Conference (10th CNC), 22-10-2008, Beijing, China. From left: Mark L Wahlqvist, Qipei Liu, Faji Zhao, juesheng Li, Xiaoshu Chen, Jingfan Gu

10th China Nutrition Conference (10th CNC), 22-10-2008, Beijing, China. From left: Mark L Wahlqvist, Faji Zhao, juesheng Li, Xiaoshu Chen, Jingfan Gu, Zhiqian He, Xuecun Chen

First IUNS-CNS Chinese Nutrition Leadership Training Workshop, 06-06-2008, Hangzhou, China. From left, front row: Duo Li, Mark L Wahlqvist, Jingfan Gu, Keyou Ge, Yiyong Cheng

Commentary

Food and nutrition science: The new paradigm

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Nutrition was invented in the early 19th century as a biochemical science that reduces foods into significant chemical constituents. Ever since then, the teaching and practice of nutrition has been based on this conceptual framework, or paradigm. The examples given here are dietary guidelines and other food guides. The first guides issued up to the middle of the last century were designed to help prevent nutrient deficiencies, promote growth, and ensure plentiful diets. These recommended foods then thought to contain adequate proteins, fats, carbohydrates, vitamins, minerals and trace elements, as well as dietary energy. At a time of accelerating industrial production of food, they were generally effective. Within the second half of the century, guides were developed and changed to counter the rapid rise in heart disease in the USA, the UK, and other high-income countries. These recommended less foods of all types high in fat, saturated fat, cholesterol and sodium, more 'complex carbohydrates', and fruit and vegetables rich in microconstituents. They probably had some limited effect. In this century and now, dominant guides have been changed again in attempts to counter what has become pandemic obesity and diabetes. These recommend less food high in saturated fat, sugar and sodium, with less emphasis on total fat and more on sugar. They are not effective. All these guides are derived from and governed by the biochemical paradigm of nutrition science. This was once useful, but now should be discarded as obsolete except for addressing deficiencies. Here, a new paradigm is proposed.

Key Words: food and nutrition science, paradigms, The New Nutrition Science, the NOVA food system, Brazilian food guides

INTRODUCTION

This commentary is in seven parts. First, the invention of nutrition as a biochemical science. Then, food guides based on this paradigm issued in the last century and up to date. Then, what paradigms are. Then, the 2005 New Nutrition Science; the NOVA food classification as from 2009; and the 2014 Brazilian food guide. Finally, the proposed paradigm for food and nutrition science is defined, with purposes and principles.

What is the definition and purpose of 'nutrition science'? This often seems vague. A definition in *The Shorter Oxford Dictionary* is: 'The branch of science that deals with (esp. human) nutrients and nutrition', and 'nutritionist' is defined as 'an expert in or student of (esp. human) nutrients and nutrition'. These definitions are practically circular.

The teaching and practice of conventional nutrition science has become dominated by the USA and to a lesser extent the UK and some other industrialised countries, and also since the creation of the United Nations by relevant UN agencies. A standard textbook with 107 authors (all but 6 from North America or Europe),[1] states that 'nutrition is an ever-changing science' but does not define 'nutrition' or state its purpose, which can however be deduced from its 65 chapters within 760 large-format pages. The first 36 have sections on energy physiology; macronutrients; fat-soluble vitamins; water-soluble vitamins; and minerals and trace elements. The other 29 have sections on the life-cycle; physiology and patho-physiology; nutrition and chronic diseases; food, nutrition and pathophysiology; international nutrition, and 'emerging issues' such as biotechnology, functional foods, and the human genome.

While these books do not explicitly state what nutrition science is, or what it does, or why, they make apparent that it is not just a basic science, but is also concerned with health, in the medical sense of preventing and treating various physical human disorders, disabilities and diseases. Its nature is indicated in the preface to another textbook (whose 24 authors are all but 4 from Europe or North America), commissioned by the UK Nutrition Society primarily for students of nutrition. It states: '*The study of human nutrition needs a solid base in the physiology and biochemistry of human metabolism*'.[2] This also does not explain why, or indicate alternatives.

Thus identified, nutrition focuses on nutrients, which is to say some of the very many bioactive chemical constituents within foods, as well as dietary energy. Its main evident practical purpose is to promote adequate

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feeding, and to prevent and treat specified physical conditions of humans believed to be caused by deficient, inadequate, or unbalanced diets. It is not concerned with good health other than absence of ill-health, nor with well-being. As such it is largely an adjunct of the predominant practice of medicine.

With time, nutrition science in this 'classic' form has become more complex, but its general nature remains essentially the same. Its biochemical conceptual framework, which to say paradigm, continues to focus on nutrients and energy, and so continues to govern food guides, the example given here. Guides include dietary guidelines, food composition tables, reports, statements, pamphlets, posters, specifications, and other educational material, published above all by national governments and international and national organisations, periodically as from the early 20th century.

THE MID-19TH CENTURY
THE INVENTION OF NUTRITION SCIENCE

For many centuries, food and its role in health was seen differently. Beginning in Egypt around 4,000 BCE, China around 2500 BCE, and then India, Greece, the Arab world, and Western Europe up until the 18th and early 19th centuries CE, interest in and study and practice of food and health was part of the qualitative natural philosophy of the good life well led, identified in Greece as for example by Plato as *diaita* - dietetics. Taking various forms in different civilisations, dietetics fosters the good health and well-being of all aspects of humans: physical, mental, emotional, moral and spiritual. What, how, when, where and with whom habitually to eat is part of the whole dietetic wise way of living.[3-5]

But in the mid-19th century, dietetics was displaced. Following the work of Antoine-Laurent de Lavoisier, François Magendie, and others, the German chemist Justus von Liebig (1803-1873) narrowed and isolated nutrition as a quantitative biochemical discipline, turning it into a 'hard' science, following the discoveries that food can be reduced into various identified measurable chemical macro- and micro-constituents needed for growth, health and life.[6]

Of these, von Liebig regarded protein as 'the only true nutrient', because it promotes and accelerates growth. He devised artificial 'NPK' fertiliser. He invented the first commercial artificial baby formula based on cows' milk, which is far higher in protein than breastmilk and therefore in his view superior, together with flour of wheat, malt and (later) peas, and potassium bicarbonate, as in effect fertiliser for infants. He developed the first commercial meat extract as a restorative and 'super-food'. He was a successful entrepreneur, and a rough competitor who wrecked the reputations of natural philosophers and other rivals, while gratifying the ruling classes.[7]

With his followers, von Liebig blazoned 'physiological chemistry' as he called it, as essential for plant, animal and human breeding. His vision was that this could harness and master nature, and would engineer the food systems of industrialising countries. He believed that his formulations and his science could transform the human race. His work accelerated the industrial and agricultural revolutions.

Von Liebig's special importance to governments was that high-protein diets bred and sustained big tall strong men fit to endure land wars, then almost incessant within Europe. His importance to industry was that he valorised the production of beef, milk and other dairy products, all high in protein and also fat. With the growth of railways, the invention of disassembly lines,[8] and increased use of freezing, chilling, canning and bottling, these became immense enterprises most of all in the USA, gaining the power to change life on earth, as they have done. Protein of animal origin is still emphasised.[9]

The eclipse of German science as a result of the two world wars made Justus von Liebig internationally less well-known than Louis Pasteur, but his impact on human life has been just as great. His concept of nutrition as a biochemical science is still dominant, as 'classic' nutrition. Until recently it has rarely been questioned inside the nutrition profession.

THE EARLY AND MID-20TH CENTURY
EAT AND DRINK MORE

All sciences meant to be useful, such as nutrition, have contexts and needs, which in time, change. In the early and mid-20th century, up to the 1970s, food guides, designed to generate public policies and actions, and food tables, for use by nutritionists and dietitians, continued to categorise foods in terms of specified chemical constituents. They primarily addressed two contexts and needs critical at that time. One was nutritional deficiencies, inadequacies and imbalances, then and now endemic in the global South, and then also common in low-income families in industrialised countries such as the USA and the UK.[6] The other, as in the 19th century, was war, especially the two world wars and the need to promote population growth and strength, and to survive, work, and fight. Food rationing, introduced in the UK in both world wars, helped to maintain national good health.[10,11]

In this period, food guides were published in the USA, the UK and increasingly in many other countries, and internationally by the League of Nations and then by United Nations agencies. They recommended groups of foods seen to be good sources of dietary energy, protein, carbohydrate, fat, minerals, and as from the 1920s, vitamins.

The concept of types of foods grouped according to their comparative contribution of energy and macro- and micronutrients, pioneered in the late 19th century in the USA by chemist Wilbur Atwater, became a feature of official US food guides throughout the 20th century and to date.[12] A 1917 guide issued by the US Department of Agriculture[13] stated on food selection: *'Perhaps as easy a way as any… is to group the different kinds according to their uses in the body and then to make sure that all the groups are represented regularly in meals… 1. Fruits and vegetables; 2. meats and other protein-rich foods; 3. cereals and other starchy foods; 4. sweets; and 5. fatty foods'*. On sugar, the guide said: 'Unless small amounts of very sweet materials – sugar itself, syrup or honey – are used, the diet is liable to be lacking in it'. The five

food groups, which included sweets and also fatty foods, were retained in USDA publications throughout the 1920s,[14] and the system of foods grouped according to their relative content of specified nutrients has been used ever since.

International food guides were issued by the League of Nations. In 1936 a League report stated specifically of whole (full-fat) cow's milk: *'Milk is the nearest approach we possess to an ideal food…It contains all the materials essential for the growth and maintenance of life… Milk should represent a large proportion of the diet of every age'*.[15] In the UK John Boyd Orr, who became founding director-general of the UN Food and Agriculture Organization in 1946, wrote in 1940 commenting on a 1937 UK Ministry of Health report on nutrition:[16] '*The Advisory Committee on Nutrition … has strongly recommended that every child should have at least 1½ pints of milk a day. The unanimity of the importance of milk is of special interest… It is… rich in first-class protein, minerals, and most of the vitamins*'.[17]

In the UK, national nutrition was supported in 1940 at the beginning of the Second World War by publication of an official report containing tables, with the accurate title *The Chemical Composition of Foods*.[18] Its preface began: '*The nutritional and dietetic treatment of disease, as well as research into problems of human nutrition, demand an exact knowledge of the chemical composition of food*'.

The 1960 edition dropped the word 'chemical' from the title, as have later editions co-published by the Royal Society of Chemistry. These tables are regularly updated and elaborated, and are now greatly expanded, but retain their original form and basic structure. All official tables published to date specify what is seen to be the relevant acknowledged chemical composition of foods. They, and versions compiled in the USA, devised in and for temperate industrialised countries, have been adopted or adapted and developed in other countries and by the UN Food and Agriculture Organization.[19] Among nutritionists and dietitians they have quasi-biblical status.

In 1954 the UK Ministry of Health made further specific recommendations for consumption of whole cow's milk, which because of its protein and fat content remained seen as an ideal food. These were for children and adolescents from 1 to 21, 1 pint a day, expectant mothers 2 pints a day, and all other adults half a pint a day.[20]

Seven editions of the Manual of Nutrition published by the UK Ministry of Food between 1945 and 1970 began by defining carbohydrates, fats and protein, and stated: *'See that the building foods are well represented. Make sure that the protective foods are included. Let appetite determine how much of the energy foods are to be added'*. For children: '*Bread, and particularly cake made with fat, sugar, milk and eggs, are excellent as concentrated sources of calories'*.[21] The focus of the Manual was on home cooking. There was no discussion of industrial food processing.

Three groups of 'body building', 'protective' and 'energy' foods were identified in 'food chart' posters issued by the UK government's Central Office of Information in the 1940s to the 1950s. A wartime poster included a group of 'energy foods', symbolised by a hammer labelled ENERGY that 'provide food for the body', listing sugar, dried fruit, honey, cheese, butter, margarine, dripping, suet, and lard, as well as potatoes, bread, flour, oatmeal, rice, sago, bacon, and ham. The message was: 'eat something from each group every day'.[22]

In the US, guidance from its Department of Agriculture continued to group foods according to their relative contribution of chemical constituents. In 1958 its *Food for Fitness: A Daily Food Guide* grouped the 'Basic Four'. These were a milk group, for protein and fat (2 to 4 cups, depending on age); a meat group,– beef, veal, pork, lamb, poultry, fish, eggs, with as alternatives beans, peas and nuts, also for protein and fat (2 or more servings); a vegetable/fruit group, for vitamins and minerals (4 or more servings); and a bread/cereal group, wholegrain, fortified or restored, for carbohydrates (four or more servings), 'plus other foods as needed to complete meals and to provide additional food energy and other food values'. The USDA retained versions of the 'Basic Four' for the next 22 years.[12,14]

Up to the 1970s, the general policy of official dietary guidelines in the USA, the UK, and some other industrialised countries, with collaborative policies and actions, especially from the farming and food manufacturing industries, was to address deficiencies and undernutrition by helping to enable the mass of populations to have plenty to eat. This was good news for and so supported by the intensive agriculture and food processing industries and the fast food and soft drink businesses. Emphasis was given to meat, milk, dairy and other animal foods and products, good sources of protein and fat as 'building foods'; and to cereals, cereal products, other starchy foods, and sugar and sugary foods, good sources of carbohydrate and dietary energy, as 'energy foods'; and fruits and vegetables, good sources of vitamins and minerals, as 'protective foods'.

Food guides published up to the 1970s generally addressed 'home-makers'. The nutrition scientists responsible for food guides were quite often funded by or advisors to industry, or employed by industry before, at the time or later, as they are now. In any case, they usually seemed to have no special knowledge of food technology and processing, or agriculture, or the preparation and cooking of meals, or of dietary patterns, or of food culture. Their main attention was on the known and recognised chemical components of whole foods. Problems included the inconvenient fact that dishes and foods as for example pies, stews and sausages, and biscuits, cakes and ice-cream, contain combinations of protein, carbohydrate and/or fat, and that the contents of meals cannot be readily quantified. The guides paid little attention to food eaten out of the home, despite the rise of fast food and drink outlets at first in the US.[23] They also generally ignored processing other than freezing, chilling, fermenting, canning and bottling, despite the increased and extensive use of technological processes such as hydrolysis, extrusion, and partial hydrogenation of oils in the manufacture of margarine and many commercial baked goods. Food additives, then and now were omitted, and thought to be only of toxicological concern.

Compliance with the recommendations of these guides often helped to reduce rates of deficiency and under-nutrition in many countries.[24] In the USA, UK and other high-income countries, national incentives and subsidies given to the intensive agriculture and food manufacturing industries helped to develop food systems and supplies. On the whole, the guides issued up to the later 20th century were evidently valuable.

THE LATER 20TH CENTURY

EAT AND DRINK LESS

Later and roughly into the late 1980s and early 1990s, food guides issued in the USA, the UK, other high-income countries, and by UN agencies, altered the findings of previous guides. The emphasis became not so much on recommending more food seen as healthy, as on less food seen as unhealthy.[25] This was bad news for and so opposed by the intensive agriculture businesses and the leading food processing and fast food and soft drink industries, which as from the 1980s became increasingly transnational. They developed and strengthened their front, representative and associated organisations.[26]

The main reason for the new emphasis was a new crisis. Heart disease, previously uncommon, had become epidemic in the USA and various fully industrialised countries, and was predicted to become common world-wide. Some attention was given to obesity, prevalence of which was rising notably in these countries. Of 100 such reports published between 1961 and 1991, 93 recommended consuming less fat, 85 less saturated fat, 47 less dietary cholesterol, and 82 less sugar, with few or none disagreeing, and of those that specified food, 51 recommended less fatty meat or meat products, 53 less full-fat milk, 50 less butter and 27 fewer eggs, with few or none disagreeing.[25] Later reports in this period often set quantified targets, such as 10 per cent or less of dietary energy from saturated fat, with implications for all types of food that are sources of saturated fat.[27]

A 1982 report from the World Health Organization,[28] emphasised vegetables, fruits, cereals and beans, as containing 'good quality' protein and as low in fat, saturated fat, sugar, sodium and dietary energy; lean and low-fat meat and dairy products; and less use of oils and fats. Foods to 'de-emphasise' were with two exceptions whole foods: high-fat meats, whole milk, cream, cheese, and eggs, 'commercially baked products' (unspecified), and alcoholic drinks.

These and later guidelines were driven above all by the theories of the US physiologist Ancel Keys of the University of Minnesota, featured on the cover of *Time* magazine in January 1961, whose influence became dominant worldwide in the mid- and later 20th century. His personality was like that of Justus von Liebig: he controlled colleagues, excoriated rivals, and charmed government officials. He led the Seven Countries Study of middle-aged men mostly in rural villages in the USA, Finland, the Netherlands, Italy, Yugoslavia, Greece (Crete and Corfu) and Japan, initially completed in the late 1960s, the largest epidemiological study of its type until then carried out. This confirmed his opinion that the chief dietary cause of cardiovascular disease was diets high in saturated fat and cholesterol, and he convinced his colleagues, professional bodies, and the governments of the USA and then other countries.[29,30] This made nutrition more mystifying outside the nutrition profession, because these substances cannot be seen or sensed.

One response from the intensive agriculture and food processing industries to what had become a worldwide expert consensus, endorsed by governments and relevant UN agencies, was to reformulate many foods and products. Cows and pigs were bred to be less fat. Lower and low-fat milk became more available. Many food products became made in versions lower in fat, but often higher in sugar. Consumption of fat and saturated fat decreased in various industrialised countries.[31]

The recommendations of these guides are generally agreed to have helped to reduce heart disease in the USA, UK and other high-income countries, which was also often successfully treated with drugs and surgery. The prevalence of obesity increased, including in middle-income countries. Preoccupation with heart disease meant that these guides were of little if any use for deficiencies. So the guides issued in the late 20th century were of limited value.

INTO THE 21ST CENTURY

PANDEMIC OBESITY AND DIABETES

As from around the 1990s the priority switched again. The context was and remains in the 2020s the crises of obesity and diabetes. Most conspicuously since the 1980s, prevalence of overweight and obesity had continued to rise in high-income countries and then especially in middle-income countries and even in many low-income countries in Latin America, Asia and Africa, as had prevalence of diabetes. Both still rise; in the highest-income countries obesity may now be reaching a peak at up to or around 30-40% of the adult population.

The *Dietary Guidelines for Americans* (DGA), issued every five years since 1980s, is the national official food guide that is internationally most influential, together with reports from relevant UN agencies. It is published jointly by the US Department of Health and Human Services, and the US Department of Agriculture which is responsible for the US agriculture and also food manufacturing industries. The DGA have never stated or even suggested that these industries are responsible for producing and manufacturing unhealthy food. Their guidance is addressed only to people as consumers.[12]

All editions of the DGA have maintained the biochemical paradigm of 'classic' nutrition science, continuing to group foods in terms of their relative content of the chemical constituents known or thought to affect human physical disorders, disabilities and diseases. They pay little attention to how foods are produced and processed, or to meals, or to dietary patterns. The US government has ruled that sustainability is out of scope.[32] Well-being is largely limited to photographs of people eating and looking happy that accompany the text.

The 2015 DGA, for 2015-2020, specifies in its third guideline: 'Limit calories from added sugars and saturated fats and reduce sodium intake'. The 2020 DGA, for 2020-2025,[33] has been issued when obesity and diabetes have been commonly identified as out-of-control epidemics in the US, and as pandemics. This DGA has

changed only incrementally from previous issues. It has more on meals and some on dietary patterns, but there is still little on how foods are processed. Its fourth guideline is: 'Limit foods and beverages higher in added sugars, saturated fat, and sodium, and limit alcoholic beverages'. Thus sugar (as added sugars) and salt are highlighted as well as saturated fat. The recommendation on cholesterol disappears. The guidance of these and the previous DGAs remain based on dietary energy and various chemical constituents of food.

There is little or no evidence that the DGAs issued since 2000 have improved the health of the US population. Now, there is a crisis of confidence, as evident in the separate report set up by the US government to advise the 2020 DGA. This states: *'The typical American dietary pattern is not currently nor has it ever been aligned with recommendations issued by the Dietary Guidelines for Americans since their inception in 1980'*.[34] Scholars in the USA point out failures of the DGA, or have a broader view.[31,32,35]

CURRENT FOOD GUIDES ARE OBSOLETE

Disquiet concerning the current stance of the US government evident in what its 2020 DGA says and what it omits, is expressed in the separate advisory report, in statements not included in the approved report. One is: *'Burgers and sandwiches, casseroles, pizza, snacks and sweets, and beverages (other than milk and 100% juice) contribute 50-60% of total energy intake. For the total population, the top 5 contributors to energy intakes include burgers and sandwiches; desserts and sweet snacks; rice, pasta and other grain-based dishes; sweetened beverages; and chops, crackers and savory snacks'*.[34] Another is: *'The food… components to limit… consumed in excess… [come from] sweetened beverages… desserts and sweet snacks, candy and sugars, breakfast cereals and bars, burgers and sandwiches, higher fat dairy products, food items that are predominantly fat… and mixed dishes, such as pizza…. Snacking is more prevalent—almost universal'*.[34] Most of these foods, listed but not characterised in the advisory report, are mass-produced, branded, processed and packaged products.

The US DGAs are given here as examples, because of their influence and power. Food guides issued throughout the world continued to follow the biochemical paradigm,[25] including the 1990 UN WHO report on Diet, Nutrition and the Prevention of Chronic Diseases.[27] The most recent 2003 UN WHO/FAO report with the same title has goals expressed as percentages of dietary energy for total fat, saturated fats, polyunsaturated fats, trans-fats, monounsaturated fats, carbohydrate, protein, cholesterol, sodium chloride, dietary fibre, and non-starch polysaccharides, and also fruits and vegetables specified as rich sources of micronutrients and 'bioactive substances'.[36]

In 2011 the WHO set up its Nutrition Guidance Expert Advisory Group (NUGAG), which has issued or is preparing reports on total fat intake and weight gain; saturated fatty acids and trans-fatty acids and cardiovascular disease; polyunsaturated fatty acids; sugars, weight gain and dental caries; non-sugar sweeteners; carbohydrates; sodium and cardiovascular disease, and potassium and cardiovascular disease. Finally, a NUGAG report on healthy dietary patterns seems to be one of those in preparation.[37]

Table 1. Summary
Current food guides are obsolete

Key reasons why, are that they:
-Fail to achieve their stated objectives
-Assume that nutrition is biochemical, quasi-medical
-Reduce foods into separate chemical constituents
-Merge whole foods with processed products
-Use language fully understood only by professionals
-Identify people mainly as individual consumers
-Focus only on some disorders, disabilities, diseases
-Recommend artificial or unrealistic diets
-Neglect or omit fresh meals, families, ways of eating
-Say little about society, economy, the environment
-Isolate humans from the natural and physical worlds
-Ignore technological changes, future generations

Since around the 1990s, food guides in the form of dietary guidelines have virtually all failed in their objectives. In this time obesity and the closely related disease of diabetes have become pandemic, and rates of other diet-related disorders and diseases have increased in many countries.[38]

Table 1 indicates the failings of almost all current food guides. Some are as follows. They have little if any relevance for deficiencies. They often merge fresh foods with processed products. In effect they recommend artificial or unrealistic diets. They pay little attention to dietary patterns and meals. They address food consumption, not production, and people as consumers, not as citizens. They say nothing about the impact of technology as used by corporations to formulate what are now identified as ultra-processed food products. They omit food additives. They are silent on the effect of dietary quality on susceptibility to and severity of infectious diseases, including those caused by coronaviruses. They mostly ignore the social, cultural, economic, political and environmental determinants of food systems and supplies and thus of what people eat and drink. Most have little or nothing to say about food as this relates to and affects the living and physical world and the biosphere.

Additionally, once foods are seen as wholes, it is obviously irrational to identify food with a small number of its over 26,000 chemical constituents.[39] Many of these that are ignored in current food guides are now known to affect human functions and health.

Also, the foods specified in food guides are biased towards those available in temperate countries. Food composition tables may not give values for indigenous tropical plant foods, many not exported and some higher in nutrients than temperate versions,[40] although work on this is being done by the UN Food and Agriculture Organization.[19] Further, they neglect or ignore herbs and spices, commonly added in preparation of meals; while insignificant in dietary energy, many are concentrated sources of bioactive compounds known or thought to protect against diseases or to enhance good health.[41]

Above all, they persist in reducing foods into chemical constituents, and so remain entrenched in the biochemical paradigm of 'classic' nutrition science.

One partial exception is the first Brazilian official national food guide issued in 2005 and in a new edition in 2008.[42] It is written in plain language that any attentive reader can understand. It sets out the principles on which it is based; these include recognition of food culture and the need for environmental sustainability. Its guidelines are for people as citizens and family members, and separately also for government and industry, and for health professionals. Its first five guidelines address healthy meals and foods; grains, roots and tubers; fruits, vegetables and salads; beans; and meat, milk and dairy products and eggs. Information about food constituents is separated in boxed text.

Times, needs and foods have changed. Relevant crises and changing circumstances now include the obesity and diabetes pandemics, economic globalisation, transnational food corporations, the depletion of non-renewable resources, the rapid increase of an ageing and physically bigger world population, increased food insecurity, worldwide pollution, plundered ecosystems, biodiversity collapse, climate disruption, and the Covid-19 pandemic, with its variants. In this age of the Anthropocene,[43] the nature of food systems and supplies has become or is becoming transformed.

Also, nutrition science and therefore food guides need to address good health and well-being as well as disorders, disabilities and diseases; to be concerned with whole human beings, mind, body and spirit; to include future generations; and to see humans as part of the living and physical world. All in all, the guides issued in the 21st century from now on, need to be based on a new paradigm

WHAT PARADIGMS ARE

Paradigms express ideology. They encapsulate ideas, principles, theories, and concepts that are created and shaped by beliefs, discoveries, knowledge, intentions, assumptions, objectives, and perceived priorities and needs. They define and govern fields of human activity.

Prevailing paradigms, which practitioners may take for granted or be unaware of, determine, circumscribe and control accepted thinking, standards and research, and so shape policies and actions. As ways of viewing what is seen as reality, they are like maps.[44,45]

No science of any type is absolute or final. There is no such thing as one complete, objective account of nature. Sciences are dynamic. They are not merely logical or mathematical. They begin with ideas, which are then developed and tested by observations, experiments, new discoveries and experience. No science is 'the final truth'.

Paradigms may remain intact for centuries, or be accepted as dogmas, or may be formulated relatively recently, or be fragile or contested. Like maps, paradigms need to be redrawn when they are evidently inadequate or misleading. If anomalies, confusions, paradoxes or crises emerge and persist, or circumstances and needs change, paradigm 'shifts' are liable to be implied or proposed, as here, and as now often in the literature, after which the prevailing paradigm may be adjusted, reshaped or eventually fully replaced. The proposed shifts are like redrawn maps. The actual shifts, as in what become reshaped new standard textbooks, teaching, practice, policies and actions, are like explorations that enable the creation of new societies.

Replacement of established paradigms that have governed conventional thinking and practice for a long time is likely to be a gradual process. A competing paradigm may be conceived and proposed, as here, which is then discussed, adjusted, tested and publicised, and seen by a growing number and then by a critical mass of influential organisations and scholars as more valuable, plausible, relevant or useful. Then the prevailing paradigm is indeed liable to be replaced by a whole new paradigm, complete with definition and principles, which prevails.[46,47] This all takes time. Max Planck, the originator of quantum theory, observed, perhaps pessimistically:[48]

'A great scientific truth does not triumph by convincing its opponents and making them see the light, but rather because its opponents eventually die, and a new generation grows up that is familiar with it'.

PROPOSED PARADIGM SHIFT 1
THE NEW NUTRITION SCIENCE

What now follows is an account of three initiatives which together propose or indicate a whole new paradigm for food and nutrition science. All three have been devised and developed as teamwork and have become used and supported by thousands of scholars, commentators and organisations and citizens in many countries. Two of them are currently widely recognised and publicised on the internet and in social, broadcast, and print media. All build on supportive or convergent work that has been done previously and recently, some listed in the acknowledgements that follow this commentary.

The biochemical paradigm governing nutrition science was challenged in 2002, at conferences in Australia and New Zealand whose presentations were organised by Mark Wahlqvist, then President of the International Union of Nutritional Sciences. The opening plenary session was developed and published as two papers in this journal.[44,45] The introduction to the first paper stated: *'A new nutrition is emerging,.. whose ideology places humans within nature, and whose theses make a wider frame, able to fit the world as we can discern it. The new nutrition gives equal value to personal, population and planetary health, with all that implies*'. The papers proposed a new map for nutrition science.

Then in April 2005 a new conceptual framework for nutrition, amounting to a proposed paradigm shift, was formulated, agreed and published as the New Nutrition Science project. This built on and synthesised previous work.[49,51]

A total of 25 scholars and authors, including Mark Wahlqvist, Ricardo Uauy and Ibrahim Elmadfa, the three successive presidents of the International Union of Nutritional Sciences (IUNS) between 2001 and 2012, formed a panel for a three-day workshop at the University of Giessen, Germany, under the aegis of IUNS. As well as food and nutrition scientists its membership included people qualified or practicing in epidemiology,

population health, natural history, agriculture, economics, philosophy and medicine. Several members were advisors to national governments or United Nations agencies. The workshop was chaired by Christopher Beauman of the European Bank for Reconstruction and Development. As explained by Mark Wahlqvist:[52]

'*The task has been to … formulate a new definition, new goals and a new conceptual framework for a science fully equipped to meet the challenges and the opportunities of the world in which we now live, in this new century. This I believe to be the most urgent and exciting task now facing our profession'.*

The proceedings of the workshop were published in September 2005 in a whole special issue of the journal *Public Health Nutrition.*[53-55] The key product of the workshop is *The Giessen Declaration,*[56] agreed and signed by all workshop members. This defines nutrition suitable for the 21st century as a social and environmental as well as a biological and behavioural science. A passage in the Declaration summarises the 21st century context and needs:

'The human species has now moved from a time in history when the science of nutrition, and food and nutrition policy, has been principally concerned with personal and population health and with the exploitation, production and consumption of food and associated resources, to a new period. Now all relevant sciences, including that of nutrition, should and will be principally concerned with the cultivation, conservation and sustenance of human, living and physical resources all together; and so with the health of the biosphere'.

Another passage specifies principles:

'The overall principles that should guide nutrition science are ethical in nature. Its principles should also be guided by the philosophies of co-responsibility and sustainability, by the life-course and human rights approaches, and by understanding of evolution, history and ecology'.

The *Declaration* and accompanying papers on the New Nutrition Science amount to the expansion of nutrition to a central part of public health on a grand scale, making it perennially positive, flexible and valuable. The New Nutrition is co-equally committed to the human, living and physical worlds, to present and future generations, and to good health and well-being as well as to prevention of disorders, disabilities and diseases. Its scope is global. It is holistic, discerning the big picture, of food systems and supplies and how these shape dietary patterns – what societies, families and people habitually eat and drink. Ricardo Uauy stated in the special issue of *Public Health Nutrition*:[57]

'The most important and urgent issues that confront nutrition scientists in the twenty-first century are beyond the scope of conventionally defined human biology. We must be willing to encompass the social, economic, political and human rights dimensions of nutrition'.

In September 2005, The New Nutrition was launched in a plenary presentation at the 18th IUNS International Conference on Nutrition held in Durban, South Africa. Between 2006 and 2007 it was discussed and endorsed at workshops, meetings or conferences held in Spain (Barcelona), Australia (Hobart), China (Hangzhou), Canada (Montreal), Sweden (Stockholm), Chile (Santiago), and Brazil (Rio de Janeiro). An additional economic dimension was proposed at the Australian meeting, which was discussed and approved by the New Nutrition council.

Figure 1. The New Nutrition dimensions agreed in 2006.[58] *Reproduced with permission.*

Public Health Nutrition, a textbook published in 2007, stated:[59]

The New Nutrition Science is… a holistic paradigm because it is informed by analysis… based on integrating social (including cultural, economic and political) dimensions, with the 'classical' biological (biochemical, physiological, medical) dimension'.

New Nutrition concepts were incorporated in declarations prepared and agreed at two further conferences. In 2008 *The Hyderabad Declaration. Public Health in the 21st Century*, which also included the concept of fundamental and elemental public health needed for impoverished populations and communities, was presented at the opening conference of the Public Health Foundation of India and published in its proceedings.[60] In 2009 the *Istanbul Declaration, Health The First Human Right*, was agreed at the annual conference of the World Federation of Public Health Associations.[61] The language of relevant United Nations agencies is now usually consistent with *The Giessen Declaration.*

Table 2 indicates the qualities of the New Nutrition Science. It fosters the meaning of health as stated by the World Health Organization: 'Complete physical, mental and social well-being and not merely the absence of disease or infirmity'.[62] It also implies a systems multi-disciplinary approach taking social, economic and environmental impacts and benefits fully into account, of which impressive examples have now been prepared and published.[43,63-65]

PROPOSED PARADIGM SHIFT 2
THE NOVA FOOD CLASSIFICATION

The New Nutrition amounts to a map that includes much new identified territory. What it does not do, is show what has gone wrong with food systems and supplies and dietary patterns and specify how to put them right. This

Table 2. Proposed paradigm shift 1.[53-56]
The New Nutrition is convincing

Key reasons why, are that it:
-Establishes nutrition as central in public health
-Makes nutrition clear, integrated, effective
-Uses evolutionary, ecological, ethical principles
-Embraces the living, natural and physical worlds
-Includes personal, population, planetary health
-Integrates biological, social, environmental science
-Addresses well-being, not only disorders, diseases
-Involves anthropocentric impacts on life on earth
-Examines the past and is for generations to come
-Respects established food systems, dietary patterns
-Recognises dietetics as the good life well led
-Fits the facts and faces the future of the 21st century

was done soon afterwards by the NOVA food classification system. NOVA was originated in the global South, in Brazil, first published internationally in 2009,[66,67] and adopted in the current second Brazilian official national food guide issued in 2014, discussed below.[68]

Boyd Swinburn, lead author of the 2019 *Lancet* report on obesity, under-nutrition and climate change,[64] states: *'One major paradigm shift in the last decade has been the NOVA classification of foods based on the level of processing rather than nutrient composition'*.[65] The key perception of NOVA is that the nature, purpose and extent of food processing has become the main dietary determinant of states of health and well-being, and of obesity and diabetes and various disagreeable, dangerous or deadly disorders, disabilities and diseases of most if not all human vital organs and systems.[67,69,70]

Since the 1980s, food systems and supplies and dietary patterns all over the world have been or are becoming transformed. The driver is what is done to food before it is purchased and consumed, which is to say, food processing.[67,69,70]

Almost all food is processed in some way before it is consumed. NOVA classifies all foods into four groups: unprocessed and minimally processed; processed culinary ingredients; processed; and ultra-processed.[67,69,70] Most individual processes are benign or neutral. Some are malign, such as the partial hydrogenation of oils.[71] Minimal processes such as drying, peeling or chopping have various functions such as to preserve whole food or make it palatable. Processed culinary ingredients (such as refined oils, sugar and salt) are rarely if ever consumed by themselves and are prepared with unprocessed and minimally processed food to make dishes and meals. Processed foods are modified from their original whole form by processes such as canning or curing.

Ultra-processed products are not modified foods. Their ingredients are chemically altered proteins, fats and carbohydrates made into imitations of real foods by sophisticated use of chemical additives. Many are high both in sugar and fat, a combination very rare in nature. Most include little or no whole food. They are designed to be convenient (durable, ready-to-consume anytime, anywhere), delicious (often hyper-palatable, and even addictive), and highly profitable while relatively affordable (low-cost ingredients), and liable to displace all other foods. Most are branded and made by transnational corporations that market them aggressively, often with colossal budgets.[67,69,70]

The matrix or structure of whole and minimally processed foods contain very many constituents, in proportions that have evolved to ensure the function and health of living organisms. When foods are ultra-processed their structure is destroyed. The chemical constituents including additives they contain are artificially formulated and have no natural balance. Many of these have no equivalents in nature, so humans are not evolved and are unlikely to be adapted to metabolise them.[72,73]

Production and consumption of ultra-processed food has greatly increased especially since the 1980s. It now amounts to a half or more of the dietary energy consumed in high-income countries such as the USA, Canada and the UK. Rates are rapidly rising in many lower-income countries.[67,69,70] In the same period, rates of obesity and diabetes are and are becoming much higher.

Studies including meta-analyses carried out in Australia, Brazil, Canada, Chile, China, Colombia, France, Italy, Korea, Mexico, Portugal, Spain, Taiwan, the UK, the USA and other countries show that ultra-processed food causes diets to deteriorate and is a cause of overweight, obesity, and diabetes. Other conditions and diseases implicated include hyperuricemia, hypertension, cerebrovascular disease, coronary heart disease, breast cancer, non-alcoholic liver disease, renal function decline, Crohn's disease, and cardiovascular, cerebrovascular and all-cause mortality.

What effect ultra-processed foods have on the human microbiome, the bacterial separate while inter-related ecosystem within the gut, often now identified as a vital organ, is not yet well-known. But it is likely to be harmful. So far, epidemiologist Tim Spector of King's College, London, thinks that the harm is done by various additives. He says: '*The data are probably best for artificial sweeteners that are derived from things like paraffin and the petrol industry, so our bodies and our microbes are not used to breaking them down. But it could be other stuff, like the enzymes you don't get on the label, or emulsifiers. There are few studies on emulsifiers, and nearly all in animals, but they show that you get reduced diversity and more inflammatory microbes… I think it's safe to say that ultra-processed foods are bad for your gut microbes and we should avoid eating them regularly'*.[74]

Now there is also good evidence that some ultra-processed foods are addictive. Ashley Gearhardt of the University of Michigan, a specialist in this field, says: *'Ultra-processed foods are created in ways that parallel the development of addictive drugs, including the inclusion of an unnaturally high dose of rewarding ingredients that are rapidly absorbed into the system and enhanced through additives. As with addictive drugs, some (but not all) individuals exhibit an addictive pattern of consumption marked by diminished control over intake, intense cravings, and an inability to cut down despite negative consequences'*.[75]

'The need to reshape global food processing', a statement addressed to the September 2021 United

Nations Food Systems Summit, concludes:[38]

The totality of evidence… shows beyond reasonable doubt that increased consumption of ultra-processed foods is a major contributor to the pandemic of obesity and related diseases. There is also mounting evidence of the harmful effects of the ultra-processed food industry on the planet, through its global demand for cheap ingredients that destroy forests and savannahs, its displacement of sustainable farming, and its resource-intensive manufacturing and packaging'.

Ultra-processing has been prominently featured on the internet and on broadcast and print media in many countries. In 2021 a *Newsweek* cover feature denounced ultra-processed food. Its cover picture, headlined TOXIC FOOD, is of a cheeseburger with a label: '*WARNING. Ultra-processed Food Raises the Risk of Diabetes, Cancer, Heart Disease, Obesity, and Dying of COVID-19'*.[76] Also in 2021, physician, microbiologist and broadcaster Chris van Tulleken showed on BBC1, the main BBC television channel, the effect on him of eating for a month a diet of 80% ultra-processed food, as now eaten by one in five people in the UK.[77] After the month was over, he reported poor sleep, heartburn, unhappy feelings, anxiety, sluggishness, and a low libido. He also had piles from constipation. He gained 6.5 kilograms. He said: 'I felt ten years older'.

Attention to ultra-processed food has tended to obscure the main message of NOVA. This is positive. Dietary patterns based on diverse and varied unprocessed and minimally processed food and processed culinary ingredients with some processed foods, made into freshly prepared meals, maintain good health and well-being and protect against disease. Long-established dietary patterns such as the northern Mediterranean diet, those of various Asian countries such as within China, Korea, India and Japan, and countries and regions within Latin America and Europe, are examples.

Table 3 indicates the qualities of the NOVA food classification system. Some are as follows. It transcends the chemical classification of foods. It explains the explosive world-wide rise of obesity and diabetes that has taken place especially since the 1980s. It is easy to understand. It enables comparable studies examining the effects of ultra-processed food on health and disease to be carried out all over the world. It proves beyond reasonable doubt that ultra-processed foods are pathogenic. It shifts responsibility for obesity, diabetes and other conditions from consumers to producers. It applies world-wide and to people of all ages and classes. It identifies healthy food patterns, based on whole and minimally processed foods made into freshly prepared meals together with processed culinary ingredients and some processed foods.

Table 3. Proposed paradigm shift 2.[67,69,70]
The NOVA food classification is comprehensive

Key reasons why, are that it:
-Transcends identification of food with chemicals
-Changes the focus of nutrition to production
-Accounts for pandemic obesity and diabetes
-Endorses essential and benign food processing
-Validates whole and minimally processed food
-Is supported worldwide by scientific investigation
-Shows that ultra-processed food is pathogenic
-Applies to all countries and societies
-Recommends foods to which humans are adapted
-Identifies fresh dishes and meals as healthy
-Includes complete human body systems
-Works throughout life from infancy to old age

PROPOSED PARADIGM SHIFT 3
THE BRAZILIAN FOOD GUIDE

The outstanding exception to the food guides based on 'classic' nutrition science is the 152-page *Dietary Guidelines for the Brazilian Population*, issued in November 2014, and available in Portuguese, Spanish, and English.[68] As stated by the Food and Agriculture Organization of the United Nations, it contains 'a full set of information and recommendations for all Brazilians… to promote the health and well-being of people, families, communities, and the whole Brazilian population, now and in future'.[78]

This *Guide* was commissioned, overseen, approved, and published by the federal Ministry of Health, after a three-year process in partnership with the Pan American Health Organization. Successive drafts were evaluated in many workshops with public health professionals and civil society organizations from all 26 Brazilian States. Drafts were circulated on-line for comments; 3,125 were received, compiled and considered, and then the final draft was completed. Over 60,000 copies of the printed versions have been distributed to health professionals, health centres, schools, hospitals and other places throughout the country.[68,79,80]

The *Guide* begins by stating the principles on which it is based. The first is: 'Diet is more than intake of nutrients', which is to say: *'Diet…refers to how foods are combined and prepared in the form of meals, how these meals are eaten, and also to cultural and social dimensions of food choices, food preparation and modes of eating'*. The second is: 'Dietary recommendations need to be tuned to their times'. An example is: *'Rates of obesity and diabetes have been rapidly increasing'* in Brazil and many other middle-income countries. The third is: '*Healthy diets derive from socially and environmentally sustainable food systems'*. This points out that: *'In most parts of the world, the means of production and distribution of food has been changing, in ways that jeopardise the equitable distribution of wealth, the autonomy of farmers, the generation of employment… and the protection of natural resources and biodiversity, as well as production of safe and healthy food'*. The fourth is: 'Different sources of knowledge inform sound dietary advice'. As well as experimental and clinical studies: *'Traditional dietary patterns, evolved and adapted often for very many generations… are… an essential natural experiment that needs to inform guidance on nutrition and on health in all senses'*. Fifth is: 'Dietary guidelines broaden autonomy in food choices'. This is developed by knowledge that: *'Many factors – whether of a physical, economic, political, cultural or social nature – can positively or negatively influence eating patterns'*.[68]

The focus of the *Guide* is not on chemical constituents of foods, but on foods, meals and dietary patterns. Its recommendations are not in effect for artificial diets never normally consumed, made up from foods whose constituents have been variously calculated to be adequate or optimal, but from actual diets habitually consumed by around one-fifth of the Brazilian population. These were analysed from the official national Household Budget Survey of the diets consumed in all Brazilian regions, urban and rural areas, and all social classes. They typically are based on the long-established Brazilian staples of rice, beans and greens, with some meat, together with salads and fruits. Eight various breakfasts and lunches and dinners are shown in photographs, together with examples of beans, cereals, roots and tubers, vegetables, fruits, nuts, milk and cheese, meat, and water.[68]

The four recommendations of the *Guide* for citizens and family members are as follows. First: *'Make natural or minimally processed foods the basis of your diet'*. Second: '*Use oils, salt and sugar in small amounts for seasoning and cooking foods'*. Third: *'Limit the use of processed foods, consuming them in small quantities… as part of meals based on natural or minimally processed foods'*. Fourth: *'Avoid ultra-processed foods'*. The *Guide* also has an overall 'golden rule': *'Always prefer natural or minimally processed foods and freshly made dishes and meals to ultra-processed foods'*.[68]

The *Guide* has been widely celebrated. In the USA, the headline in *The Nation* was 'Welcome to Brazil, where a food revolution is changing the way people eat'.[81] *The Atlantic* summarised: *'A revolutionary nutrition strategy based around a few simple rules: Eat food. Mostly plants that are native to your country. And absolutely nothing "ultra-processed" '*.[82] The news website *Vox* headlined: 'Brazil has the best nutritional guidelines in the world', and gave its context: *'The way we talk about nutrition in this country is absurd. And you only need to look as far as Brazil to understand why. Yesterday, a US-government appointed scientific panel released a 600-page report that will inform America's new dietary guidelines…They take a rather punitive approach to food, reducing it to its nutrient parts and emphasising its relationship to obesity. Food is removed from the context of family and society and taken into the lab or clinic. Brazil… does exactly the opposite. Their national guidelines don't dwell on nutrients, calories, or weight loss... Instead, they focus on meals, and encourage citizens to simply cook whole foods at home, and to be critical of the seductive marketing practices of Big Food'*.[83]

Recommendations on ultra-processed food products are included in guides issued by the Pan American Health Organization of the World Health Organization. They are also featured in the national guides of some other Latin American countries, and Malaysia, Israel and France. 'Choose minimally processed foods instead of ultra-processed foods' is one of the ten dietary pattern recommendations of the 2021 American Heart Association *Scientific Statement on Dietary Guidance to Improve Cardiovascular Health.* In 2021, the EASL-Lancet Liver Commission made similar recommendations for preventing liver diseases.[38,84-86]

The transnational corporations that make and sell most ultra-processed food dislike the *Guide*. It was flat-out attacked in September 2020. A 'technical note' from officials at the Brazilian Ministry of Agriculture, Livestock and Supply, supported by the Brazilian Food Industry Association, leaked to the media. The note claimed that the *Guide* was 'one of the worst on the planet' and called on the Ministry of Health urgently to review it, and to cut out its recommendation to avoid consumption of ultra-processed food products. In response, 33 scholars from the USA, Canada, the UK, Australia, New Zealand, South Africa, Mexico, and Chile, many of whom advise their governments, wrote to the Minister of Agriculture confirming that the 'technical note' had no valid foundation.[87]

Most impressive was the response from The Alliance for Adequate and Healthy Food and Eating, a coalition of over 30 civil society organizations. The Alliance mobilised 349 organizations and 45,983 citizens from all over Brazil. These were concerned for or engaged in human rights, food security and autonomy, child, family and public health, society, culture, employment, retailing, catering, cooking, farming, ecology, and other interests, occupations and professions. In support of the *Guide*, the Alliance stated: '*The increasing number of people affected by chronic non-communicable diseases associated with the consumption of ultra-processed foods… is not only Brazilian but is global… Chronic diseases are associated with the severity and lethality of Covid-19. This further reinforces… the need for equitable, resilient and sustainable food systems [which] … should aim first and only at the health of people and the planet'*. Faced with this demonstration of nationwide solidarity, the Minister of Agriculture repudiated the 'technical note' and confirmed that nutritional issues are the responsibility of the Ministry of Health.[87]

In 2021 the Ministry of Health published a further food guide, the 262-page *Dietary Guidelines for Brazilian Children Under 2 Years of Age*, in Portuguese and English. This emphasises the vital importance of extended exclusive breastfeeding. It incorporates the philosophy, findings and recommendations of the 2014 *Guide.*[88]

Table 4 indicates the qualities of the 2014 Brazilian food guide. Some are as follows. It states its principles. The dietary pattern it recommends corresponds to that of a proportion of the Brazilian population. It is based on freshly prepared meals. It is for everybody, designed to be read and used by professionals and by people as citizens and family members. It is universal and can be readily adjusted for all other countries and regions. It promotes positive good health and well-being, not just avoidance of disorders, disabilities and diseases. It involves society, economics, and politics, and the living and physical worlds. It is in the great tradition of public health.

THE NEW PARADIGM

In order to shift a paradigm that is no longer useful, and to establish a new paradigm that addresses evident crises, meets current needs, and accommodates known facts, its name, definition, purpose and principles need to be agreed. Those offered below are developed from those

Table 4. Proposed paradigm shift 3.[68,79,80]
The Brazilian food guide is compelling

Key reasons why, are that it:
-Stands on explicit, timely, rational, ethical principles
-Recommends diets actually eaten within populations
-Uses concepts and language accessible to everybody
-Addresses people as citizens and family members
-Separates whole, processed, ultra-processed foods
-Embraces good health, well-being, not only ill-health
-Celebrates freshly prepared meals and social eating
-Proposes action on existing knowledge and wisdom
-Considers society, economics, politics, environment
-Integrates human, living, natural, physical worlds
-Mobilises professional, civil society organisations
-Applies everywhere to everybody, now and in future

specified for the New Nutrition Science as a result of the series of workshops, conferences and other meetings after the conference in South Africa (Durban), held in Germany (Giessen), Spain (Barcelona), Australia (Hobart), China (Hangzhou), Canada (Montreal). Sweden (Stockholm), Chile (Santiago), Brazil (Rio de Janeiro), India (Hyderabad) and Turkey (Istanbul), and subsequent work. They also incorporate the philosophy of the NOVA food classification and the 2014 Brazilian food guide. They are open for debate and discussion at conferences and meetings and in the literature.

Once reviewed and finally agreed, they should be declared and published in all contexts as the governing statements of the theory and practice of the science. These include the constitutions and preambles of all relevant professional societies, research centres, textbooks and journals; policy statements including all types of food guide developed and published by United Nations agencies, other relevant international and national organisations, and governments at all levels from national to municipal.

The proposed name for the science is new. It recognises that nutrition is about food and not only nutrients. It restores the concept of alimentation – food as consumed – which remains part of the name of the discipline in romance languages, as for example *alimentação e nutrição* in Portuguese, and *Lebensmittel und Ernährung* in German. The science has various components. For example, much of what is now termed 'nutrition' is actually clinical nutrition, one of whose concerns is the alleviation and treatment of nutritional deficiencies.

Like all organised rational human activity, food and nutrition science needs to be based on explicit principles. These give context, structure and meaning, govern and guide thought and action, and create purpose, force and focus to research and practice. They evolve. They can be explored and challenged at any time. They are not forever true or false; they are more or less relevant and valuable, depending on circumstances and needs. They answer 'why?' and 'what for?' questions. Below are 21 that have been discussed and agreed at the meetings mentioned above, developed since then and for this commentary. More can be added. They can be adapted for different regions, countries, times and situations.[53-56,89]

Name
Food and nutrition science.

Definition
Food and nutrition science is concerned with the physical, mental, emotional, moral and spiritual health and well-being of humans, within the living and physical worlds and the biosphere. It is a central part of personal, public and planetary health. It is a biological, behavioural, cultural, economic, political and environmental discipline.

Purpose
Food and nutrition science studies and guides the nature and interactions of food systems and supplies, dietary patterns, meals, foods and drinks, and nutrients. It protects and promotes good health and well-being, and thus contributes to a world in which present and future generations fulfil their human potential, are protected from disorders and diseases, live wisely and well, and develop, sustain and enjoy an increasingly rich and diverse environment. It is the basis for policies and actions that identify, create, conserve, protect and develop rational, sustainable and equitable local, national and global food systems, so as to sustain the health, well-being and integrity of humanity, and that of the living and physical worlds and the biosphere.

Principles
General
Humanity is moving out of the era of reckless exploitation, production, and consumption. Now, human responsibilities include preservation, protection, conservation and sustenance.

Food and nutrition science follows evolutionary, ethical, and ecological principles, respects history, culture and tradition, affirms human rights, and helps to preserve and protect the human, living and physical worlds.

Food and nutrition science supports everybody to fulfil their human potential, to live in the best of health, and to develop, sustain and enjoy increasingly diverse human, living and physical environments.

Evolutionary
Food and nutrition theory, policy and practice, respects the evolutionary processes that over millions of years have shaped the evolution of hominid species and eventually *Homo sapiens*.

Ethical
A special responsibility of food and nutrition science is to hand on to future generations an improved human, living and physical environment: healthy people, healthy populations, and a healthy planet.

Ecological
All relevant sciences, including that of food and nutrition, are concerned with the cultivation, conservation and sustenance of human, living and physical resources and the biosphere.

Historical
Food and nutrition practices consistently followed in different cultures and times in history are probably valid. They do not need proof to be accepted, adopted or adapted, but disproof to be rejected.

Biological
Nutrition defined as a biological science cannot slow or stop disease epidemics. The social, cultural, economic, political and environmental determinants of epidemics are outside its scope.

Nourishment
The single nutritional factor that most protects human health lifelong is extended exclusive breastfeeding. Breastfeeding is also emotionally vital, socially valuable, and environmentally sound.

The main dietary determinant of health is the extent to which foods are processed. Meals mainly made with unprocessed and minimally processed foods are healthy. Ultra-processed products are harmful.

As a rule, natural foods that are whole or modified by simple processes are healthy. Artificial food products formulated by sophisticated techniques are unhealthy and should be avoided.

The best nourishment is from commensal freshly prepared meals. Good company and pleasant surroundings increase enjoyment and well-being and enhance all aspects of human health.

Social
It is essential to acknowledge the vast rapid recent global, national and local social developments, and their basic and underlying driving forces, to prevent disease and sustain human well-being and health.

Cultural
Good husbandry, sound nutrition, and great gastronomy are inextricably linked. Home cooking supplies nourishment, family and social well-being and cohesion, good local relationships, and autonomy.

Economic
Food subsidies in rich countries, and tariffs imposed on food from poor countries, damage human health, social fabric, and the environment, and are a cause of intractable epidemic diseases.

Political
Basic causes of epidemics include decisions increasingly taken beyond democratic process. Effective action to control and prevent disease requires revised and renewed structures of governance at all levels.

Environmental
Rational food and nutrition policies and actions protect global renewable and non-renewable resources and sustain renewable resources. They do not depend on non-renewable resources.

Traditional
Indigenous and traditional food systems known or reliably considered to be beneficial to human health, with light environmental impact, should be preserved, protected, reinstated and developed.

Agricultural
Mixed farming systems that support the natural fertility of the soil by sustainable methods, with minimal chemical inputs, are ecologically and environmentally sound and produce healthy food.

Food systems
Healthy dietary patterns derive from socially, economically and environmentally sustainable food systems, that are based on established cultures, prevailing climate, and existing terrain.

Public health
Food and nutrition science is a central part of public health. As such it addresses the fundamental and elemental health conditions of impoverished populations and communities.

CONCLUSION
What still remains conventional or 'classic' nutrition, with its biochemical paradigm, has governed the science for close to two centuries. It is a remnant of an ideology invented in Europe in which humans were seen as superior to and separate from the living world and physical environment, free recklessly to ravage resources many of which are irreplaceable. All this must now end. In any case, initiatives designed to improve food and health are now evidently beyond its scope. It has minimal value, is no longer fit for purpose, and should be set aside as obsolete and replaced.

Proposals have recently or currently been made, as here, that imply or propose shifts of the paradigm. The still-prevailing paradigm is beginning to shift among a gradually increasing number of national governments, professional leaders, and in the United Nations. Here is the introduction to the 2021 UN Food Systems Summit:[90]

'Food is more than just what we eat. The ways in which we produce, process and consume food touch every aspect of life on this planet. Food is the foundation of our cultures, our economies, and our relationship with the natural world, and has the power to bring us together as families, communities and nations'.

What so far has been missing, is a name, definition, purposes and principles for food and nutrition science. These are offered here. The future was envisioned by the Spanish/Venezuelan José María Bengoa, a founder of public health nutrition, at the First World Congress on Public Health Nutrition in Barcelona, in September 2006. Then in his 94th year, he said:[91]

'One can glimpse a great expansion in the horizons of the science of nutrition… We are getting closer and closer, like a great magic wheel, to the ideas that the Greeks held about dietetics – as the dominion of life itself, both in the biological and social sense. It seems as if we are redefining nutrition as the beginning and end of life itself'.

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Review Article

Intermittent fasting may optimize intestinal microbiota, adipocyte status and metabolic health

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The aim of this review is to provide an overview of the present association between Intermittent Fasting (IF), the Gut Microbiota (GM), and the adipocyte with respect to Metabolic Health (MH). A search was carried out through Dialnet, Scielo, Web of Science, Redalyc and PubMed, using keywords such as: "intermittent fasting", "time-restricted feeding", "gut microbiota" and "Metabolic Health". Intermittent fasting (IF) regimens promote weight loss, therefore contributing to improved metabolic health. IF beneficially participates in the modulation of the intestinal microbiome, allowing a continuous interaction with nutrients to be digested and shaping the intestinal immune responses during the development of cardiovascular disease, blood pressure and diabetes mellitus through metabolic activities.

Key Words: intermittent fasting, intestinal microbiota, metabolic health

INTRODUCTION

The globalization of the Western lifestyle, through the so-called epidemiological transition has allowed noncommunicable diseases (cardiovascular disease, diabetes mellitus and dyslipidemias) to be responsible for approximately 67% of mortality worldwide. The increase in adipose tissue is associated with a set of metabolic disorders, such as the so called Metabolic Health (MH).[1,2] The MH is characterized by a series of metabolic disorders or abnormalities that together are considered a risk factor for the development of diabetes mellitus and cardiovascular diseases, being the most important characteristics of this syndrome are: abdominal obesity, atherogenic dyslipidemia, hypertension, insulin resistance and prothrombotic-inflammatory situations.[1-5] If the subject presents three of the five factors according to the Adult Treatment Panel III 2014 diagnostic criteria, they will be considered with MH, these are: Waist circumference: >102 centimeters (cm) in men and >88 cm in women; blood pressure: >130/85 mmHg, fasting capillary glycemia: ≥100 mg/dL; high-density lipoprotein: <40 mg/dL in men and >50 mg/dL in women; hypertriglyceridemia: plasma triglycerides ≥150 mg/dL.[6] Poor control over dietary patterns (excess meals during the day or prolonged fasting >15 hours (h)) leads to an altered circadian rhythm, which translates into metabolic dysregulation, an altered metabolic homeostasis and increased cardiometabolic risks in these patients.[7-10] In this context, health care providers have proposed dietary improvement and structured lifestyle interventions as the first line of defense. However, due to patients poor or non-adherence to changes in their food quality and quantity, low or no physical activity and the promotion of weight loss with low-calorie diets are inadequate; since these strategies are difficult to maintain for prolonged periods of time, therefore, its effectiveness for the treatment of MH is limited. As an alternative strategy, intermittent fasting (IF) has been used to achieve progressive weight loss in obese people.[11] The alternative dietary weight loss strategies that involve restricting energy intake to certain periods of the day or prolonging the fasting interval between meals (intermittent energy restriction, IER). These strategies include intermittent fasting (IF; >60% energy restriction on 2-3 days per week, or on alternate days) and time-restricted feeding (TRF; limiting the daily period of food intake to 8-10 h or less on most days of the week).[12] Intermittent fasting is a pattern of eating in which there are alternating periods of eating and a defined phase of prolonged fasting. IF can be defined as, a voluntary abstinence from food and drink for specific periods, in addition to recurring. In IF, the subject's participation is voluntary.[11-14] The intestinal microbiota (GM) plays imp-

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ortant roles in our organism as it has a reciprocal relationship with the circadian rhythm and eating habits. Food intake alters the inherent diurnal rhythm of the intestinal microbiome, the food content itself and feeding times play a key role in this process.[15,16] IF promotes browning of white adipose tissue and decreases obesity through modification of the intestinal microflora.[17] Time-restricted feeding contributes to the decrease of various obesogenic microorganisms and increases the proliferation of bacteria with protective functions against obesity.[18] Based on the above, the objective of this review is to provide an overview of the present relationship between Intermittent Fasting (IF) regimens and Intestinal Microbiota (GM) in patients with Metabolic Health (MH), through the search for information in the following computer resources: Web of Science, Pubmed, Redalyc, Scielo, Dialnet and Google Scholar; using and with keywords including intestinal microbiota, intermittent fasting, metabolic health and intestinal dysbiosis.

PHYSIOLOGICAL BASES OF FASTING

During the fasting phase, a coordinated alteration of metabolic and transcriptional mechanisms is induced. After 12 to 36 h of fasting, the body has decreased blood glucose concentration, decreased liver glycogen stores, and hepatic production of fat-derived ketone bodies, or ketones, which are used as energy for the brain.[19,20] Glucose sensitive neurons respond by activating sympathetic neurons, for example, norepinephrine released in the stomach allows the stimulation of ghrelin secretion, which affects the release of growth hormone, thereby maintaining plasma glucose concentration.[21,22] The fasting biological environment causes an elevated glucagon/insulin ratio, which facilitates the mobilization of free fatty acids towards the liver, being a sufficient stimulus to form ketone bodies (30% of free fatty acids present in adipose tissue are converted in the liver to ketone bodies). In the physiological condition of prolonged fasting for several days, ketones become the preferred fuel source of the brain, providing between 65-70% of its energy needs, becoming a more efficient source of energy in the muscles and brain, improving bioenergetics, as well as the connective activity of neurons.[20] When insulinemia is low, the liver forms ketone bodies from acetyl-CoA. Under these conditions, lipolysis is active and increases in adipose tissue, releasing increasing amounts of fatty acids. A quantity of these substances is taken up by the liver, where β-oxidation is activated, with a consequent production of acetyl-CoA. As the anabolic pathways that acetyl-CoA could follow are blocked, such as the synthesis of fatty acids and cholesterol due to the absence of insulin and the consequent deactivation of the regulatory enzymes of these processes, acetyl-CoA is channeled into the formation ketone bodies (Acetoacetate, Beta-hydroxybutyrate and Acetone).[23] Beta-hydroxybutyrate (BHB) is involved in signaling functions by inducing transcription of brain-derived neurotrophic factor derivatives (BDNF), which is a regulator of neuronal function that stimulates mitochondrial biogenesis, maintains the synaptic structure, regulates the production and survival of new neurons, and increases their resistance to injury and disease. Fasting induces peroxisome proliferator activated receptor gamma 1 alpha protein expression (PGC-1 alpha), involved in the modulation of genes associated with the metabolism of carbohydrates and fatty acids. Also, fasting suppresses inflammation by reducing the expression of proinflammatory cytokines (Interleukin 6; IL-6, and Tumor Necrosis Factor α; TNF-α).[23] The stimulation of gluconeogenesis is manifested through a negative nitrogen balance because during the first five days of fasting about 75 g of protein can be catabolized daily. Glycemia decreases during fasting, reaching a plateau around the third day, this fall is due to the depletion of hepatic glycogen and the delay of gluconeogenesis (Figure 1), derived from this, it remains low for about a week. With continuous fasting, several mechanisms are produced by which glycemia is normalized (the tissues metabolize more easily fatty acids and ketone bodies; gluconeogenesis is intensified, producing 30 to 35 g/d of carbohydrates from amino acids and glycerol).[23,24] During IF, lipid metabolism is influenced by altering the hormonal activities of leptin, adiponectin, and ghrelin. Leptin is associated with a pro inflammatory state, while adiponectin is associated with increased sensitivity to insulin. Ghrelin can stimulate neurogenesis. Leptin decreases but adiponectin and ghrelin increase, these alterations are probably beneficial for the bioenergetics of neurons and the maintenance of neural pathways.[25,26] IF not only consist of not eating, but doing it at specific time intervals, that is, establishing intervals of 12 h where meals are organized and 12 h where fasting takes place, although some studies propose fasting for 16 h and eating for the remaining 8 h.[26-28]

RELATIONSHIP BETWEEN THE INTESTINAL MICROBIOTA AND METABOLIC HEALTH

The intestinal microbiota (GM) participates both in the digestion and the fermentation of complex carbohydrates, the synthesis of vitamins, the development and maturation of the immune system of the gastrointestinal mucosa, the defense against intestinal pathogens, as well as, in direct interaction with the enteric nervous system through the release of endocrine mediators in the interstitial tissue.[29] Also, GM regulates the innate and adaptive mechanisms of immune homeostasis. These bidirectional mechanisms of action are related to the epithelial and immune cells that act as an epithelial barrier, and to tolerance to the microorganisms present in the intestine. The latter are represented by the multitude of bacteria that constitute the microbiota, whether residents or transients, such as viruses, fungi, and sometimes even parasites. The intestinal mucosal epithelium participates in events of absorption, mucus production, secretion of antimicrobial peptides, various hormones, and antigen sampling. Beneath the epithelial layer, in the lamina propria, a series of innate and adaptive immune cells are located, including B cells, T cells, macrophages, dendrite cells, and innate lymphoid cells, responsible for immune responses.[30,31] The secretion of substances by GM involves short chain fatty acids (acetate, butyrate, and propionate), neurotransmitters (serotonin, dopamine, noradrenaline, gamma amino butyric acid, tryptophan, serotonin, dopamine), bile acids, hypothalamic-pituitary-adrenal axis hormones (cortisol), gastrointestinal hormones (leptin and PYY).[32] There are several neurotransmitters and hormones involved in this pro-

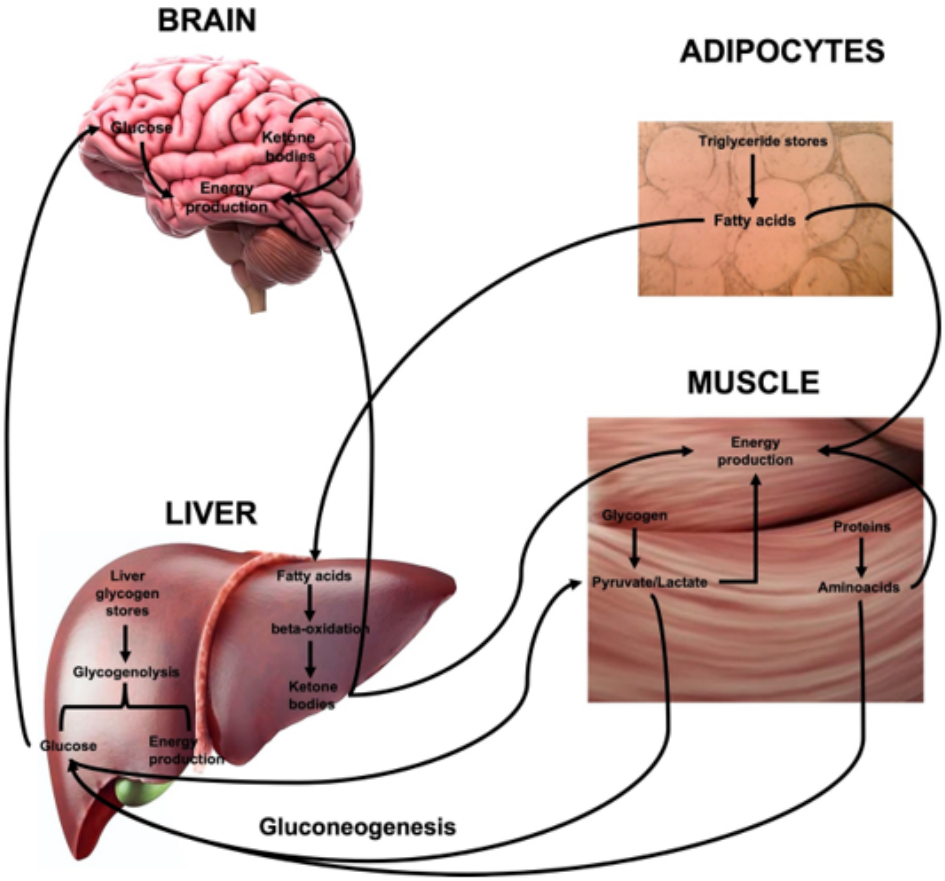

Figure 1. Metabolism and utilization of ketone bodies in intermittent fasting.

cess, there is a group called incretins (intestinal secretion of insulin) that are produced by enteroendocrine cells distributed throughout the digestive tract, from the stomach to the distal colon. Incretins enhance insulin secretion in response to glycemia, regulating it and are responsible for around 70% of the postprandial insulin concentration. The two most important are gastric inhibitory peptide (GIP) and glucagon-like peptide 1 (GLP1). Propionate modulates energy homeostasis by activating sympathetic neurons mediated by GPR41 (G41 protein-coupled receptor), in contrast to ketone bodies. The ability to modulate sympathetic outflow provides another mechanism that links the intestinal microbiota with the enteric nervous system, energy expenditure, and metabolic homeostasis.[32,33] Some bacteria producing these molecules with endocrine activity are Lactobacillus and Bacteroides. GM is fluctuating throughout growth and development, susceptible to modifications associated with diet, birth pathway and even systemic diseases. The loss of functional balance in GM is called dysbiosis, and it has been associated with MH, through GM-mediated endocrine signaling in insulin resistance and chronic inflammation. In MH, the intestinal microbiota is implicated due to its involvement in the development of obesity by increasing energy and stimulating inflammation, lipopolysaccharides (LPS) from the membranes of Gram-negative bacteria and other molecular patterns associated with microbial pathogens (PAMPs) promote inflammation, which is associated with early processes in obesity and the development of insulin resistance.[34] One of the proposed mechanisms is that the deterioration of the intestinal microbiota with a specific or modified composition leads to an increase in intestinal permeability and subsequently, to a high concentration of systemic bacterial products such as LPS (metabolic endotoxemia).[35,36] The intestinal composition of an adult is stable; however, it depends on multiple factors (environment, diet, lifestyle, and diseases). Short term and long term diets have been shown to alter the gut microbiota; in the case of short term diets, the composition of the gut microbiota reverts to its primitive state; therefore, a stable modification of the composition of the intestinal microbiota requires long term nutritional adaptations.[37,38] Insulin resistance is recognized as an indispensable part of the pathophysiology of MH, leading to compensatory hyperinsulinemia, however, this long term mechanism favors the development of obesity, contributing to the progressive failure of the beta-pancreatic cells, and triggering dysglycemia and diabetes mellitus. Nevertheless, insulin resistance does not develop homogeneously in each of the insulin sensitive tissues and their functions (regulation of lipid metabolism, cell proliferation, vascular tone, and appetite modulation). Specific changes in GM composition have been associated with the development of insulin resistance, derived from the overgrowth of microbial species with elevated short chain fatty acid fermentative activity, high pyruvate metabolism and potentiation of the pentose phosphate pathway of fatty acid biosynthesis, and glycerolipid metabolism; thus favoring the accumulation of adiposity, the development and progression of obesity and insulin resistance.[39-41] The intestinal microbiota of obese patients presents less biodiversity than that of normal weight patients, those individuals with less biodiver-

sity tend to present greater adiposity, insulin resistance, dyslipidemia and a more pronounced inflammatory phenotype compared to those with high biodiversity. The presence in the intestinal microbiota of high concentrations of Staphylococcus aureus and low concentrations of Bifidobacterium spp in childhood predict the future appearance of overweight or obesity. In relation to the predominant microbiota, changes observed in its composition and function are related to a higher risk of type 2 diabetes, which is assoaciated to an increase in the number of *Bacteroides* and *Clostridium*.[42,43] The contribution of dietary fat alters the composition of the intestinal microbiota, increasing gram-negative bacterial populations and altering the intestinal barrier function. These events lead to increased plasma concentrations of LPS and the subsequent development of a low-grade inflammatory state that facilitates the development of insulin resistance and type 2 diabetes mellitus (T2D).[44]

METABOLIC HEALTH AS A THERAPEUTIC TARGET OF INTERMITTENT FASTING

The first line of therapy for metabolic health is aggressive diet and lifestyle interventions (reducing caloric intake, adopting a healthier eating plan, and increasing physical activity), however, these interventions are insufficient to effectively control the disease, rather, it may gradually get worse, and patients are often given medications to treat their symptoms. Treatment of the metabolic health is important to prevent progression to T2D and reduce morbidity and mortality from T2D or cardiovascular diseases. Within the mechanism of participation of intermittent fasting and the relationship with the metabolic health, we can mention the mechanistic objective of rapamycin (mTOR), a serine-threonine kinase that participates as an intracellular energy sensor, stimulating the response to growth factors and increasing amino acids or glucose. Low glucose or amino acid concentrations during fasting are associated with decreased mTOR activity. Fasting regulates mTOR activity, which stimulates autophagy, cell repair, and increases mitochondrial biogenesis. Another cell mediator of interest is sirtuins (sirtuin 3), which is in the mitochondria of metabolically active tissues (heart, kidney and skeletal muscle) stimulating in response to fasting and exercise.[6,44-46] The IF cycle model of feeding with established fasting and feeding periods, generates adaptive cellular responses since cells participate in tissue specific processes of growth and plasticity during the feeding period, stress resistance and suppression of inflammation, as well as delayed aging. These functions are dependent on diet, gender, and genetic factors. Total dietary energy intake and the duration of fasting between meals favour changes in bioenergetic levels such as: NAD, ATP, and acetyl CoA. These energy transporters activate proteins that mediate cellular function and stress resistance, thereby generating neuroendocrine and adaptive responses to low glucose concentration.[47] The two main types of IF are: alternate-day fasting and time-restricted fasting. In alternate day fasting, the subset may consist of 24 h fasts followed by a 24 h period of feeding that can be performed several times a week, as noted by the 5:2 strategy, in which there are 2 fasting days mixed with 5 unrestricted days. For time-restricted fasting, variations include 16 h fasts with 8 h feeding times, and 20 h fasts with 4 h feeding times. IF is involved in the main features that compose the metabolic health spectrum, such as its participation in the control of dyslipidemias, blood pressure, obesity, and T2D.[19]

IF has been used as a dietary intervention strategy, periodic energy restriction has been shown to decrease the risks of aging and associated conditions, in addition to providing satisfactory results in terms of body weight control and metabolic health in study patients,[48] as well as cardiovascular disease and dyslipidemia. Also, IF reduces markers of systemic inflammation and oxidative stress associated with atherosclerosis. It has been reported that individuals who did not eat breakfast have a higher risk of atherosclerosis compared to those who ingested high calories at breakfast. Individuals who did not eat breakfast compared to the high caloric intake group showed unfavorable parameters: higher percentage of central obesity, body weight, body mass index, waist circumference, dyslipidemia and glycemia. Stanislawski et al (2021), mention that the intestinal microbiota plays a fundamental role in the development of obesity in addition to contributing to weight loss. Dysbiosis has been linked to the pathogenesis of obesity in both animal models and humans, derived from the mechanism involving energy homeostasis/nutrients absorption, inflammatory pathways, appetite regulation, and/or the generation of small molecules that alter metabolism. Weight loss has been shown to result in changes in the intestinal microbiota and there is evidence that the intestinal microbiota and gut-derived metabolites may be important mediators of the response to dietary energy restriction. In work conducted by these researchers they compared the weight loss produced by intermittent fasting (IF, restriction of 80% of energy intake for three nonconsecutive days per week with no restriction of intake on the intervening days) with the current standard of care dietary approach to weight loss of daily caloric restriction (DCR), both groups targeting an equivalent weekly energy deficit (34%), receiving identical exercise prescriptions and a comprehensive behavioral weight loss program based on the group.[49] In their conclusions, they demonstrated that the gut microbiota is involved in the regulation of body weight and contributes to responsiveness during a weight loss intervention. During the first three months of a lifestyle-based weight loss intervention that included an energy restricted diet and increased physical activity, the intestinal microbiota of the participants changed significantly. The initial composition of the intestinal microbiota predicted the change in waist circumference at three months and that numerous bacterial taxa were associated with improvements in weight and waist circumference measurements. This leads to the fact that the structure of the intestinal microbiota community can influence the response to weight loss efforts, which is critical to understand more fully, as the intestinal microbiota profiles can be altered through various means, such as probiotics/prebiotics, personalized diet changes, or targeting of intestinal microbiota pathways and metabolites.[49,50] Obesity: IF is effective for weight loss compared to the use of standard diets.[51,52] Another study found that it was effective for weight loss and cardiovascular health in overweight and normal

weight adults, mentioning that daily calorie restriction versus intermittent restriction is equally effective in reducing weight and fat mass.[11] In a randomized trial, they concluded that there is no superior adherence, weight loss, weight maintenance, or cardioprotection versus daily caloric restriction, although IF may be more effective for not losing lean mass.[53] Through a meta-analysis, the authors found that skipping breakfast increases the risk of over-weight/obesity by 48% in cross-sectional studies and 44% in cohort studies.[54] T2D: Two studies showed that 24 h IF (4:3), 3 times per week successfully reversed insulin resistance in patients with prediabetes or T2D, thereby reducing glycosylated hemoglobin concentrations, oxidative stress, and appetite control.[55] The study group consisted of patients were older adult, with a high percentage of women and smokers, who had consumed a diet with a higher intake of calories per day, animal protein, total fat, cholesterol, processed foods, alcoholic beverages and, on the other hand, consumed less dietary fiber, vegetables, and whole grains.[56] Another study reported that the alternate-day fasting group that underwent a 75% alternate-day caloric restriction had a 10±4% reduction in LDL and a 17±5% reduction in triglycerides after 12 weeks.[57] Another study, obese patients showed an improvement in HDL and LDL concentrations after 12 weeks of alternate day fasting combined with exercise.[57] Blood pressure: IF has been shown to reduce systolic and diastolic blood pressure. In a study of men with prediabetes, a mean reduction in systolic blood pressure of 11±4 mmHg and a reduction in diastolic blood pressure of 10±4 mmHg was observed after 5 weeks of fasting for 18 h periods.[58] Through the analysis of heart rate and blood pressure, it was concluded that those patients who perform IF have a lower frequency component in the variability of diastolic blood pressure, a marker of sympathetic tone. IF is considered to have the ability to reduce blood pressure, thus improving mortality from noncommunicable diseases such as cardiovascular diseases.[59,60] Some lifestyle modifications can promote metabolic health, such as: Sleep, there is evidence through observational studies that eating at night is associated with a reduced duration and poor quality of sleep, which can later lead to insulin resistance and thus increase the risk of obesity, diseases cardiovascular and diabetes. Altered circadian timing due to these behaviors can lead to circadian desynchronization affecting normal sleep patterns.[61-64] The metabolic disturbances associated with sleep loss may be mediated by the overgrowth of specific gut bacteria. The end products of bacterial species that grow in response to sleep loss can induce fatigue. The positive effects of intermittent fasting on sleep latency and sleep efficiency may be due to its effect on the intestinal microbiota, probiotic supplementation improves subjective sleep quality.[65] Energy consumption, most fasting regimens reduce the total number of hours available for eating and thereby may reduce overall energy consumption and risk of obesity. A dysregulation in working hours (shift or night) has shown alterations in the hormones that regulate appetite (leptin, ghrelin and xenin) that can lead to increases in total energy intake.[66-68]

ADIPOCYTE STATUS AND INTERMITTENT FASTING

The adipocyte is a cell with the ability to generate and receive information from its environment and intervene in the low-intensity chronic inflammatory process resulting from obesity. In the increase in the amount of adipose tissue, two processes are involved: the increase in size of adipocytes and the increase in the number of adipocytes. Under normal conditions, 80% of adipose tissue is in the subcutaneous cellular tissue, while visceral adipose tissue represents less than 20% of total body fat in men and approximately 6% in women. Subcutaneous abdominal fat deposits are located below the regional skin. In the lower body segment, all fatty deposits are subcutaneous; the two main sites of accumulation are the femoral and gluteal regions. Visceral adipose tissue is made up of smaller adipocytes, with less storage capacity, is more vascularized, and has greater sympathetic innervation and a large number of β3-adrenergic receptors, which facilitates greater metabolic activity.[69] There are two types of adipose tissue, and therefore two different types of adipocytes that form them: Brown or brown adipose tissue is responsible for thermogenesis; its colour is due to the large amount of hemoprotein cytochrome oxidase, and the mitochondria it possesses express high amounts of uncoupling protein (UCP), UCP that produce uncoupled oxidative phosphorylation with the consequent dissipation of energy in the form of heat; White adipose tissue is the most abundant in the adult human body and therefore the largest energy reservoir as already mentioned, in the form of triacylglycerides, coming from chylomicrons and VLDL circulating. Due to its wide distribution, it is an excellent thermal insulator and plays an important role in maintaining body temperature, being considered the main buffer system for energy balance.[70,71] White adipose tissue releases secretion products that are involved in the regulation of energy intake-expenditure and glucose homeostasis, or both (leptin, adiponectin, resistin, visfatin, acylation-stimulating protein or ASP), immune inflammatory response (TNF-α, IL-6, IL-1, C-reactive protein, serum amyloid A, haptoglobin, monocyte chemoattractant protein 1), vascular function (angiotensinogen, angiotensin, resistin), blood coagulation (PAI1, tissue factor), complement pathway (adipsin), growth factors (TGF-β), in angiogenesis (VEFG) and reproductive function.[69,72,73] The adipose tissue of obese patients is characterized by hypertrophy and hyperplasia of adipocytes and by changes in their metabolic functions, the adipocyte being the largest producer of inflammatory adipokines in these conditions.[74-77] There are several mechanisms capable of inducing inflammatory pathways: By extracellular mediators such as cytokines and lipids; Due to intracellular stress, such as stress on the endoplasmic reticulum system, understood as an increase in its functional demands induced by obesity, which causes changes in architecture, increased protein and lipid synthesis, and disturbances in energy flows and of intracellular nutrients in adipose tissue.[78] Mention is made below of some ways in which the role played by intermittent fasting and adipocytes can be seen:

Li et., mention that, while activation of beige thermogenesis is a promising approach for treatment of obesity-

associated diseases, there are currently no known pharmacological means to induce beiging in humans. Intermittent fasting is an effective and natural strategy for weight control. Every other day fasting (EODF) regimen selectively stimulates beige fat development within white adipose tissue, and dramatically ameliorates obesity, insulin resistance and hepatic steatosis. EODF treatment results in a shift in the gut microbiota composition leading to the elevation of the fermentation products acetate and lactate, and the selective upregulation of monocarboxylate transporter expression in beige cells.[79] Harney et al., establish that, intermittent fasting is a beneficial dietary treatment for obesity. A key change in subcutaneous white adipose tissue (scWAT) and visceral white adipose tissue (vWAT) depots is an increase in mitochondrial protein content after EODF. This effect is correlated with increased fatty acid synthesis enzymes in both white adipose tissue (WAT) depots but not in brown adipose tissue. EODF treatment downregulates lipolysis specifically in vWAT, mediated by a large decrease in the abundance of the catecholamine receptor (ADRB3). Enrichment analysis highlights downregulation of inflammatory collagen IV specifically in vWAT, allowing improved insulin sensitivity.[80]

CONCLUSIONS

In conclusion, we can say that intermittent fasting is a strategy to be considered today as it has wide applications not only for weight loss, but also for improving the health of people with metabolic health disorders (dyslipidemia, hypertension, T2D and/or overweight). Evidence has been found that IF participates beneficially in modulating the intestinal microbiome, allowing a continuous interaction with nutrients to digest and shape intestinal immune responses during the development of cardiovascular disease through metabolic activities. The implementation of the IF may favor the relationship between intestinal microbiota and the pathogenesis of obesity, metabolic health, and even T2D, by influencing body weight, proinflammatory activity, and insulin resistance, as well as neurodegenerative diseases. It has been found that IF collaborates in the reduction of plasma LPS, which has been recognized as a possible trigger of the systemic inflammatory response and atherosclerotic cardiovascular disease, in the same way IF is important in the increase of anti-inflammatory cytokines and in situations of metabolic oxidative stress.

AUTHOR DISCLOSURES

The authors declare no conflict of interest.

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Original Article

Skeletal muscle index and muscle attenuation with liver cirrhosis as survival prognosticators by sex

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Background and Objectives: It has been proven that skeletal muscle index (SMI) and muscle attenuation (MA) are correlated with outcomes in liver cirrhosis. However, whether there are sex differences in these factors remains unknown. We aimed to analyze the predictive ability of SMI and MA for the prognosis of cirrhotic patients of different sexes and promote computed tomography (CT) use in body composition assessment. **Methods and Study Design:** CT images taken at the 3rd lumbar vertebra from 223 patients were quantified for body composition. A Cox regression model was used to assess associations between mortality and body composition. Time-dependent receiver operating characteristic curves were calculated to evaluate the predictive ability of SMI and MA for the 1-, 3- and 5- year mortality of cirrhotic patients. **Results:** The majority of patients with liver cirrhosis were male (64.6%), and there was a weak linear correlation between SMI and MA in males (r=0.33, p<0.001). In the sex stratified multivariate Cox regression analysis, SMI in males (HR=0.95; 95% CI, 0.91-0.98; p=0.002) and MA in females (HR=0.91; 95% CI, 0.87-0.96; p<0.001) were independently associated with mortality. The areas under the curve (AUCs) of SMI (AUC=0.718) and MA (AUC=0.705) were similar in the 5-year mortality prediction of males, while in females, MA (AUC=0.797) had a stronger predictive ability than SMI (AUC=0.541). **Conclusions:** SMI in males and MA in females are independent prognostic factors for liver cirrhosis. For females, MA may be a more sensitive indicator of mortality prediction than SMI, while in males, they are equivalent.

Key Words: liver cirrhosis, body composition, skeletal muscle index, muscle attenuation, computed tomography

INTRODUCTION

Liver cirrhosis is one of the leading causes of disability and mortality worldwide,[1] accounting for approximately 2 million deaths per year worldwide.[2] In recent years, a growing number of studies have shown that skeletal muscle consumption is not only a common feature of cirrhosis and contributes significantly to worse outcomes, such as infections,[3] hepatic encephalopathy (HE) and ascites,[4,5] but also an independent predictor of survival in cirrhosis and post liver transplantation.[6-8] Muscle mass is an important predictor of liver cirrhosis.

Muscle mass has been estimated according to a variety of methods. Patients with liver cirrhosis are prone to ascites and edema, which limit the application of traditional skeletal muscle assessment methods, such as bioelectrical impedance and calf circumference measurement. Computed tomography (CT) imaging analysis is gradually being used in the muscle evaluation of patients with cirrhosis due to its objectivity, accuracy and availability. Moreover, the skeletal muscle index (SMI) is also used as a prognostic indicator for patients with liver cirrhosis, and the cutoff values for males and females are different.[9,10]

In previous studies that analyzed males and females separately, SMI was predictive of mortality in males but not in females.[6,11,12] Unfortunately, the prognostic value of SMI was predominantly evaluated in male patients, but its predictive effect in females may be obscured. Researchers have stated that skeletal muscle consumption should be interpreted differently between male and female patients with cirrhosis.[13] Therefore, a more effective indicator for assessing the prognosis of female patients with cirrhosis is needed at present.

Muscle attenuation (MA), which is measured by CT

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and expressed in Hounsfield units (HU), is associated with skeletal muscle lipid content and provides insights into pathophysiology.[14,15] Current findings suggest that MA has prognostic value in predicting overt HE and mortality in patients with cirrhosis.[16,17] Therefore, we aimed to assess the association between SMI and MA in patients with cirrhosis. Furthermore, the capability of SMI and MA to predict mortality in cirrhotic patients of different sexes is evaluated in this research.

METHODS

Study populations

Adult patients (18-80 years) who were diagnosed with liver cirrhosis by imaging examinations or biopsy results were consecutively enrolled from the Affiliated Hospital of Qingdao University between January 2015 and April 2016. Our study was performed in accordance with the Declaration of Helsinki (2000) and was approved by the ethics committee of the Affiliated Hospital of Qingdao University (QYFY WZLL 26501). A total of 898 patients were reviewed, and patients were excluded if they: (1) had primary liver carcinoma or other malignant tumors (n=560); (2) lacked CT scans within 2 weeks on index hospitalization (n=84); (3) had other chronic wasting diseases (n=11); (4) underwent liver transplantation performed during the follow-up period (n=8) or (5) were lost to follow-up (n=12). Ultimately, 223 cirrhotic patients were enrolled in this study (Figure 1).

Clinical data collection

Data for the clinical features and inspection results of all included patients, including sex, age, body mass index (BMI), etiology of cirrhosis, presence of decompensated events (ascites, HE, infection and variceal bleeding), liver function tests, coagulation tests, platelet counts and serum sodium concentration, were collected. The severity of liver disease was assessed by the model for the end-stage liver disease (MELD) score[18] and Child-Pugh score.[19] The interval between laboratory tests acquisition and CT scanning was less than 2 weeks. The primary outcome was mortality before April 30, 2021.

Evaluation of CT imaging

A plain CT scan of the abdomen is a routine examination for patients with liver cirrhosis, which can provide plentiful important information on body composition. Two sequential transverse CT images extended from L3 toward the iliac crest were analyzed using Slice-O-Matic V5.0 software (Cosmovision, Montreal, Quebec, Canada), which enables specific tissue demarcation by using HU thresholds. The CT HU thresholds were -29 to +150 for skeletal muscle area,[20] -190 to -30 for subcutaneous adipose tissue and -150 to -50 for visceral adipose tissue.[21,22] The cross-sectional area of muscle and adipose tissue was normalized for height in squared meters (cm^2/m^2), and these values were referred to as SMI, subcutaneous adipose tissue index (SATI) and visceral adipose tissue index (VATI). The visceral-to-subcutaneous ratio (VSR) was simply calculated as the visceral adipose tissue area (cm^2)/subcutaneous adipose tissue area (cm^2), which explores the distribution of abdominal adipose tissue. In addition, MA measured the mean HU of the entire skeletal muscle area,[23] which is associated with skeletal muscle lipid content.[14] All CT images were analyzed by two trained observers. For difficult cases, several specialists reached a consensus through discussion. Ultimately, the intraobserver coefficient of variation was approximately 2.3%.

Follow-up

After the patients were discharged from the hospital, routine outpatient visits were performed every 6 months, and abdominal ultrasound or CT examinations, liver function tests, tumor marker tests and other examinations were performed. Telephone interviews were regularly performed by the investigators to assess the general situation of each included patient. Survival time was defined as the interval between the first admission to our hospital for cirrhosis and death or the cutoff date (April 30, 2021), whichever came first.

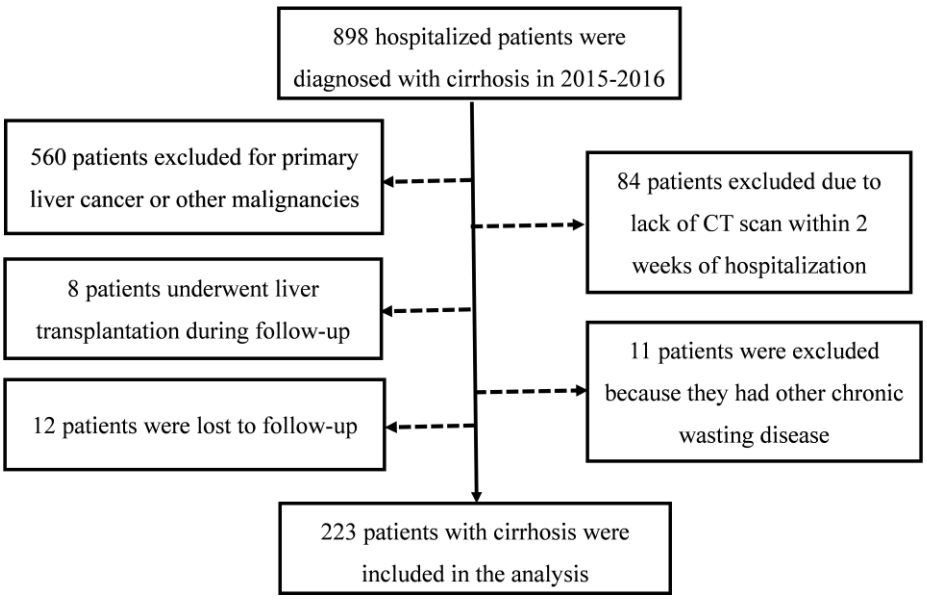

Figure 1. Flowchart of patients excluded/included in this cohort.

Statistical analysis

Data are presented as the mean standard deviation or median interquartile range (IQR), as appropriate. Differences between groups were analyzed using Wilcoxon's test for continuous variables and Pearson's χ^2 test for categorical data. Correlations between SMI, MA and age were assessed by Pearson's correlation coefficient analysis.

Univariate and multivariate analyses of overall survival were performed using Cox regression models, and the results are presented as hazard ratios (HRs) with 95% confidence intervals (CIs). Backward stepwise elimination (p>0.1) was performed to derive a more parsimonious model to identify variables that were independently associated with the outcome.

Based on the different performances of SMI and MA in male and female patients, we used time-dependent receiver operating characteristic (ROC) curves[24] to analyze the predictive value of SMI and MA for the 1-, 3- and 5-year mortality of the patients in this cohort. The area under the curve (AUC) was used to estimate the ability of the indicator to predict mortality, and the Delong test was used to compare the AUCs.[25] The optimal value of each indicator was determined by Youden's index, and MA was divided into low-MA and high-MA groups by the cutoff value in different sexes. Cumulative mortality curves were constructed using the Kaplan-Meier procedure, and the log-rank test was utilized to compare the mortality curves of the two groups.

The statistical analysis was performed using SPSS version 24.0 (IBM Corp., Armonk, NY, USA) and Medals version 18.2.1 (Ostend, Belgium). A p value <0.05 (2 sided) was considered statistically significant.

RESULTS

Baseline characteristics of the enrolled patients

Among the 223 patients who met the inclusion criteria, 144 were male (64.6%) with a mean age of 51.5±10.6 years, and 79 were women (35.4%) with an average age of 54.6±11.6 years. Hepatitis B virus (HBV) infection was the main etiology, followed by autoimmune liver disease (AILD) (20.2%) and alcohol abuse (15.7%) (Table 1).

During the hospitalization period, 125 (56.1%), 97 (43.5%), 95 (42.6%), and 45 (20.2%) patients were diagnosed with ascites, variceal bleeding, infection and HE, respectively, and there were no significant differences between males and females. The median follow-up time of the study was 65.1 (IQR, 34.7-70.9) months, and the five-year mortality rates for men and women were 38.2% and 38.0%, respectively.

Differences in body composition status according to sex

In this study, we performed sex stratification and compared baseline body composition compartments of patients with cirrhosis, including BMI, SMI, MA, VATI, SATI and VSR (Table 1). Although BMI and VATI values were not significantly different between male and female patients, SMI (51.6±8.1 vs 40.9±7.0, p<0.001), MA (39.5±7.9 vs 30.3±9.8, p<0.001) and VSR (0.90 [IQR, 0.65-1.38] vs 0.51 [IQR, 0.36-0.76], p<0.001) values were significantly higher in male than female patients. In contrast, the SATI value in male patients was significantly lower than that in female patients (30.0 [IQR, 19.1-43.65] vs 52.8 [IQR, 32.0-66.9], p<0.001).

Table 1. Baseline characteristics and body compositions in cirrhosis

Characteristics	Total (N=223)	Male (n=144)	Female (n=79)	*p*
Age, y	52.7±11.6	51.5±10.6	54.6±11.6	0.358
Etiology, n (%)				<0.001
HBV	127 (57.0)	89(61.8)	38(48.1)	
AILD	45 (20.2)	9(6.3)	37(46.8)	
Alcohol	35 (15.7)	34(23.6)	0(0.0)	
Cryptogenic/others†	16 (7.2)	12 (8.3)	4 (5.1)	0.840
Ascites, n (%)	125 (56.1)	80 (55. 6)	45 (57.0)	0.840
HE, n (%)	45 (20.2)	28 (19.4)	17 (21.5)	0.712
Variceal bleeding, n (%)	97(43.5)	66(45.8)	31(39.2)	0.342
Infection, n (%)	95 (42.6)	53 (36.8)	42 (53.2)	0.018
MELD score	11.8±4.4	12.1±4.4	11.2±4.6	0.059
Child-Pugh class, N (%)				0.976
A	79 (35.4)	51 (35.4)	28 (35.4)	
B	100 (44.8)	64 (44.4)	36 (45.6)	
C	44 (19.7)	29 (20.1)	15 (19.0)	
Albumin, g/L	31.8±7.0	32.4±7.2	30.6±6.4	0.083
Bilirubin, mmol/L	25.0 (16.5, 44.9)	25.3 (16.8, 43.8)	22.8 (16.1, 44.9)	0.921
Sodium, mmol/L	141 (139, 143)	141 (138, 143)	142 (139, 143)	0.096
Platelet, 10^9/L	89 (57, 132)	91 (55, 139)	84 (61, 123)	0.870
Body composition variables				
BMI	23.9±3.4	24.2±3.2	23.4±3.6	0.082
SMI (cm^2/m^2)	47.8±9.3	51.6±8.1	40.9±7.0	<0.001
MA (HU)	36.3±9.7	39.5±7.9	30.3±9.8	<0.001
VATI (cm^2/m^2)	25.5 (13.6, 46.4)	25.7 (13.4, 46.8)	24.1 (14.1, 43.8)	0.957
SATI (cm^2/m^2)	35.1 (22.7, 54.1)	30.0 (19.1, 43.7)	52.8 (32.0, 66.9)	<0.001
VSR	0.77 (0.47, 1.25)	0.90 (0.65, 1.38)	0.51 (0.36, 0.76)	<0.001

HBV: hepatitis B virus; AILD: autoimmune liver disease; HE: hepatic encephalopathy; MELD: model for end-stage liver disease; BMI: body mass index; SMI: skeletal muscle index; MA: muscle attenuation; VATI: visceral adipose tissue index; SATI: subcutaneous adipose tissue index; VSR: visceral to subcutaneous adipose tissue area ratio.

†Others include non-alcoholic steatohepatitis, hepatitis C virus, Wilson disease and Budd-Chiari syndrome.

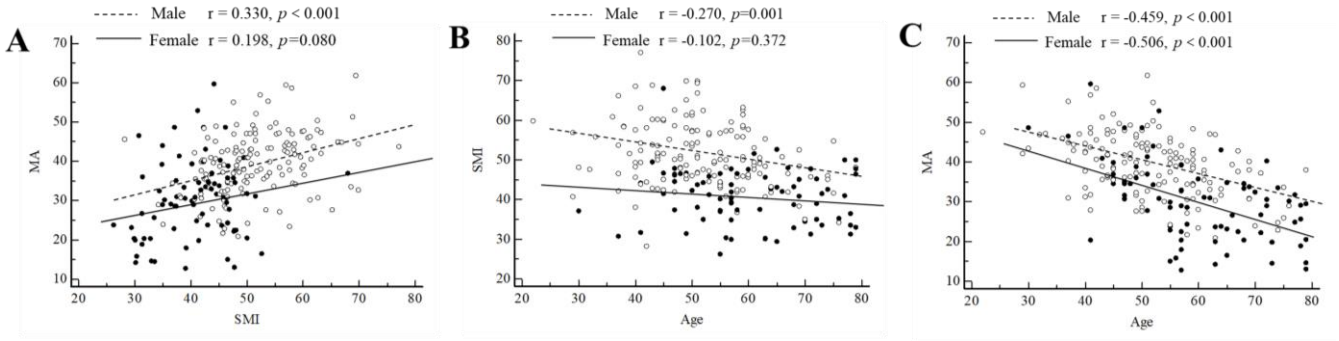

Figure 2. Scatter graphs depicting the correlations between skeletal muscle index (SMI), muscle attenuation (MA), and age according to sex.

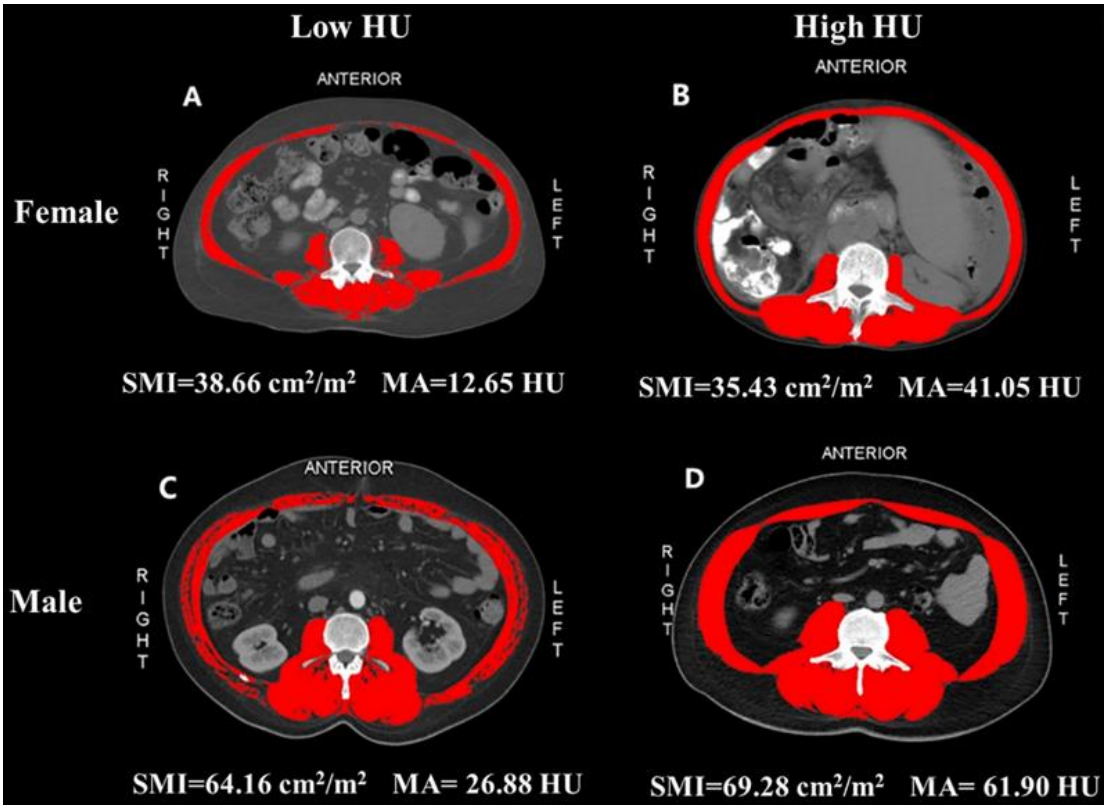

Figure 3. Comparison of two female (A, B) and two male (C, D) patients with cirrhosis. Abdominal CT images taken at the third lumbar vertebra. The red shadows show the skeletal muscle areas from -29 to +150 HU. Images A and B, images C and D have similar skeletal muscle index (SMI) values, but the muscle attenuation (MA) values are obviously different.

The correlation between SMI and MA in different sexes

There was a significant linear (p<0.001) and weak (r=0.33) relationship between SMI and MA in male patients, while a linear correlation between SMI and MA was not observed in females (Figure 2A). As shown in Figure 3, two female and two male cirrhotic patients had similar SMI values, whereas their MA values were significantly different. These results suggested that MA and SMI were two different metrics for assessing skeletal muscle, and they cannot replace each other.

Age is a known risk factor for skeletal muscle wasting. Therefore, we analyzed the correlation between SMI, MA and age. As shown in Figure 2B and 2C, in male patients, SMI (r=-0.270, p=0.001) and MA (r=-0.459, p<0.001) were negatively correlated with age. In female patients, only MA (r=-0.506, p<0.001) was negatively correlated with age.

Univariate and multivariate analyses of mortality

To quantify the effects of SMI and MA on mortality, we conducted Cox proportional hazard regression analysis, and the results from univariate and multivariate analyses are summarized in Table 2. In female patients with cirrhosis, univariate analysis indicated a correlation between MA and mortality (HR=0.91; 95% CI, 0.87-0.96; p<0.001), but SMI did not show an association with mortality (HR=0.97; 95% CI, 0.92-1.02; p=0.264). After adjusting for confounding factors, MA was still an independent factor influencing the survival of female patients (HR=0.91; 95% CI, 0.87-0.96; p<0.001). For males, MA (HR=0.93; 95% CI, 0.90-0.96; p<0.001) and SMI (HR=0.91; 95% CI, 0.87-0.96; p<0.001) were significantly associated with mortality in the univariate analysis. In multivariate Cox regression analysis, only SMI (HR=0.95; 95% CI, 0.91-0.98; p=0.002) was independently associated with mortality.

Table 2. Mortality associated factors by univariate and multivariate cox proportional-hazards analysis in female and male cirrhotic patients

Characteristics	Univariate		Multivariate	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Female patients				
Age, y	1.06 (1.02, 1.09)	0.001		
MELD score	1.20 (1.12, 1.30)	<0.001	1.22 (1.12, 1.33)	<0.001
Bilirubin, mmol/L	1.00 (1.00, 1.01)	0.002		
Sodium, mmol/L	0.96 (0.84, 1.09)	0.526		
INR	6.05 (2.83, 12.94)	<0.001		
Albumin, g/L	0.93 (0.88, 0.99)	0.016		
Ascites	2.14 (0.98, 4.65)	0.055		
HE	3.57 (1.72, 7.39)	0.001	2.51 (1.15, 5.49)	0.021
Infection	2.81 (1.29, 6.11)	0.009	3.01 (1.35, 6.70)	0.007
Variceal bleeding	1.22 (0.60, 2.48)	0.581	2.42 (1.11, 5.28)	0.027
SMI (cm^2/m^2)	0.97 (0.92, 1.02)	0.264		
MA(HU)	0.92 (0.88, 0.96)	<0.001	0.91 (0.87, 0.96)	<0.001
SATI (cm^2/m^2)	1.00 (0.98, 1.01)	0.694		
VATI (cm^2/m^2)	1.01 (1.00, 1.03)	0.078		
VSR	5.20 (1.90, 14.26)	0.001		
Male patients				
Age, y	1.04 (1.02, 1.07)	0.001	1.04 (1.02, 1.07)	0.001
MELD score	1.20 (1.13, 1.27)	<0.001	1.16 (1.09, 1.23)	<0.001
Bilirubin, mmol/L	1.00 (1.00, 1.01)	0.322		
Sodium, mmol/L	0.93 (0.87, 0.99)	0.029		
INR	3.67 (2.03, 6.63)	<0.001		
Albumin, g/L	0.94 (0.91, 0.98)	0.003		
Ascites	2.42 (1.36, 4.32)	0.003		
HE	2.86 (1.64, 4.97)	<0.001	1.99 (1.09, 3.64)	0.025
Infection	2.58 (1.53, 4.35)	<0.001	1.75 (0.98, 3.15)	0.061
Variceal bleeding	1.55 (0.92, 2.61)	0.099		
SMI (cm^2/m^2)	0.93 (0.90, 0.96)	<0.001	0.95 (0.91, 0.98)	0.002
MA(HU)	0.93 (0.90, 0.96)	<0.001		
SATI (cm^2/m^2)	0.99 (0.97, 1.00)	0.072		
VATI (cm^2/m^2)	1.00 (0.99, 1.01)	0.717		
VSR	2.26 (1.42, 3.60)	0.001		

HR: hazard ratio; MELD: model for end-stage liver disease; INR: international normalized ratio; HE: hepatic encephalopathy; SMI: skeletal muscle index; MA: muscle attenuation; SATI: subcutaneous adipose index; VATI: visceral adipose tissue index; VSR: visceral to subcutaneous adipose tissue area ratio.

In univariate and multivariate Cox proportional hazards regression models, the performance of SMI and MA in male and female patients with liver cirrhosis was different, which was an interesting discovery.

The predictive ability of SMI and MA for mortality in patients with cirrhosis

In view of the differences between SMI and MA in different sexes, we used time-dependent ROC curves to examine the predictive effectiveness of SMI and MA in predicting the 1-, 3- and 5-year mortality of cirrhotic patients of different sexes (Figure 4).

In the time-dependent ROC analysis of all 223 patients, the AUC of MA was consistently larger than that of SMI, but a significant difference was not shown until the 5-year mortality prediction (p=0.047). In males, the AUC of SMI was slightly larger than that of the MA in predicting 1-, 3- or 5-year mortality, but there was no significant difference between the two indicators. For females, the AUC of MA showed a gradually increasing trend as the observation time increased, while the AUC of SMI decreased, and the p value of these two parameters gradually decreased. The predictive ability of MA is significantly better than that of SMI for the mortality of female patients with cirrhosis.

According to the cutoff values of the ROC curves in the 5-year mortality of the different sexes, in females, MA less than 30.73 HU was defined as low-MA, with an AUC of 0.797 (95% CI, 0.692-0.879), while in males, MA <39.83 HU was defined as low MA, with an AUC of 0.705 (95% CI, 0.623-0,778).

Cumulative mortality curves were constructed using the Kaplan-Meier method (Figure 5). The mortality of the low-MA group was higher than that of the high-MA group in all patients with liver cirrhosis. In males, the 1-year, 3-year and 5-year probabilities of mortality were 19.7%, 36.6% and 54.9% in patients with low MA, compared to 6.8%, 11.0% and 21.9% with high MA, respectively. For females, the 1-, 3- and 5-year estimated mortality probabilities in patients with low and high MA were 30.0% and 2.6%, 52.5% and 5.1%, 65.0% and 10.3%, respectively (all p<0.001 by log-rank tests). Hence, patients with low MA had a higher risk of mortality.

DISCUSSION

Numerous patients with liver cirrhosis are faced with increased energy consumption, decreased appetite, and protein synthesis dysfunction, causing an imbalance in protein synthesis and consumption in the body,[26] which consequently leads to skeletal muscle consumption. Muscle tissue is more often affected in male patients, while adi-

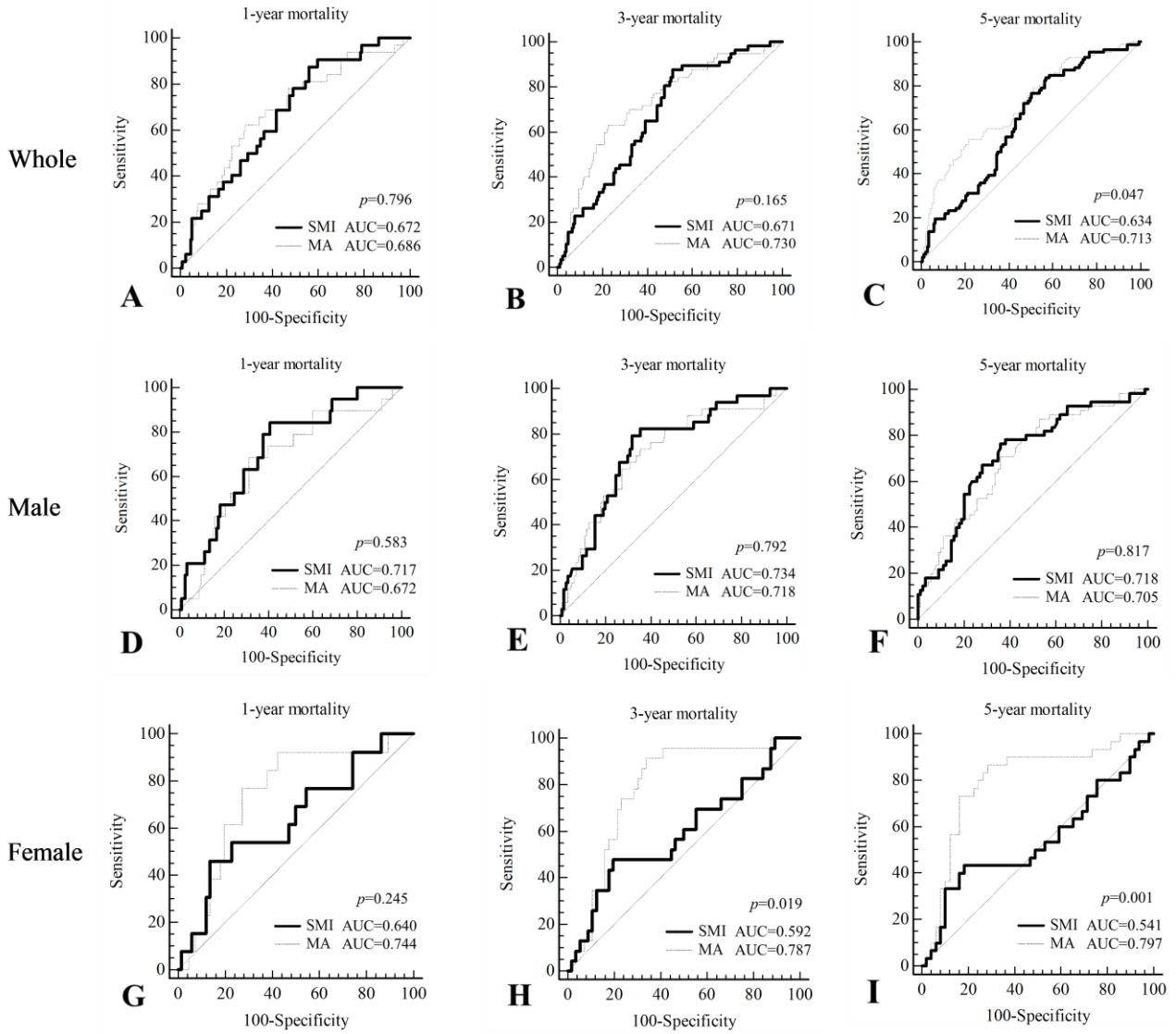

Figure 4. Predictive value of skeletal muscle index (SMI) and muscle attenuation (MA) in predicting 1-, 3- and 5-year mortality of liver cirrhosis patients in this cohort; utility of SMI and MA for predicting the prognosis of patients with cirrhosis, as determined by comparing the areas under the ROC curve.

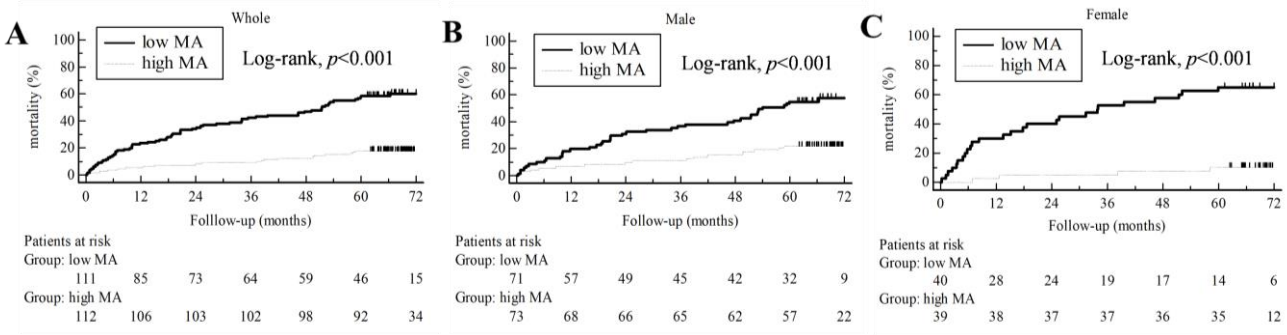

Figure 5. Kaplan-Meier curves indicating the mortality of patients with low muscle attenuation (MA) and high MA in whole (A), male (B) and female (C) patients with cirrhosis.

pose tissue is depleted more severely in females. In addition to body composition, sex differences exist for other clinical features of liver disease, such as etiology and creatinine concentration.[27] Unfortunately, sex-specific differences are often ignored in many disease studies despite obvious variability in hormonal profiles, body composition phenotype changes, differential etiologies and liver metabolism,[28-32] which emphasizes the need for sex classification in cirrhosis associated mortality studies.

By sex stratification, SMI was independently associated with mortality in male patients with cirrhosis, but this finding was inconsistent with that in female patients,[11,12] which is in line with our current results. However, the mechanism that causes sex differences in its ability to predict the mortality of cirrhotic patients is complex, but

may be related to hormone concentration. As an androgen, testosterone plays an important role in muscle synthesis. Clinical trials of testosterone have proven that the dose-dependent increases in muscle mass extend well into the supraphysiological range.[33] At the cellular level, testosterone affects the differentiation and proliferation of myocytes and regulates muscle protein turnover.[34] Serum testosterone is reduced in up to 90% of men with liver cirrhosis, and decreases with the progression of liver disease.[35] In females, the serum testosterone concentration is only one-tenth that of males, and has little effect on the production and decomposition of skeletal muscle. Therefore, it is not difficult to understand that SMI is more sensitive to the mortality prediction of male patients.

SMI and MA are two independent muscle assessment indicators, and no obvious linear correlation between them has been found in recent studies,[36] which is in line with our current findings. There are some similarities between them, and a lack of unified assessment criteria in muscle assessment makes it inevitable that some people confound the two. According to some studies, SMI and MA are defined as dichotomous variables as sarcopenia and myosteatosis, respectively.[37,38] In our analysis of 223 patients with liver cirrhosis, the AUC of MA was larger than that of SMI, although the significant differences between the two parameters did not appear until the fifth year. MA may be a better indicator than SMI, especially in long-term mortality prediction. Of note, the majority of patients with liver cirrhosis are male, furthermore, the early death caused by acute liver failure and acute gastrointestinal bleeding may influence the ability of SMI and MA to predict mortality. In the subsequent sex-stratified analyses, MA showed fine mortality prediction ability in both males and females. A recent study related to orthotopic liver transplantation (OLT) also found that myosteatosis may be a useful parameter for predicting the perioperative prognosis of OLT patients, supporting the role of myosteatosis.[39] Similar findings were disclosed in studies of other diseases. For instance, Vedder et al[40] demonstrated that myosteatosis is a stronger predictor of survival than sarcopenia in peripheral arterial occlusive disease (PAOD) in a single-center retrospective cohort study of 686 PAOD patients. In another retrospective cohort study of 228 patients with pancreatic cancer, Rollins et al[41] suggested that the presence of myosteatosis was significantly associated with systemic inflammation and reduced survival rates, while sarcopenia alone did not have a bearing on survival. In summary, MA may be a better indicator for predicting disease prognosis.

Some research has concluded that pathological variation in MA is directly associated with the accumulation of lipids,[14,42] however, little is known about the exact composition of muscle lipid components. What is known thus far is that muscle lipids comprise a variety of lipid species, including free fatty acids, diacylglycerol, triacylglycerol and phospholipids. Moreover, it may be not only the content but also the proportion of these components that may be important in the pathological effects of fat accumulation. Recent studies have revealed that the composition of lipid components in muscle may be as important as the total amount of fat per se in promoting muscle loss (both in pathology and function). For example, accumulation of diacylglycerols instead of triacylglycerol is associated with insulin resistance in non-adipose tissues.[43,44] In addition, the pathogenesis of fatty muscle infiltration has not yet been fully elucidated. The accumulation of fat in skeletal muscle is not only the result of age and continuous consumption of disease but also may be a concentrated response to the overall poor condition of patients. Excess muscle fat may be seen as stored fat to meet or predict future metabolic needs, or as fat toxicity and its cascading toxic effects (such as IR, oxidative stress, inflammation, impaired regeneration, altered protein balance).[45] Therefore, lipid composition and the mechanism of intermuscular fat deposition still need to be studied in cirrhotic patients of different sexes.

To the best of our knowledge, this is the first study to analyze the predictive ability of SMI and MA by time-dependent ROC in patients with liver cirrhosis, and we used body parameters as continuous variables for analysis to avoid the loss of information. Furthermore, evaluating the body composition of cirrhotic patients through CT imaging is objective as well as accessible, and makes it possible to simultaneously avoid the interference of fluid retention, which is also the main advantage of this study. Nonetheless, it should be noted that this is a retrospective study in a single institution, making it difficult to evaluate the preadmission diet and physical activity of these cirrhotic patients, which are also factors affecting skeletal muscle. Therefore, further research of a well-designed multicentric prospective cohort is needed in the future.

Conclusion

SMI in males and MA in females are independent prognostic factors of liver cirrhosis. For females, MA may be a more sensitive indicator of mortality prediction than SMI, especially regarding long-term prognosis, while in males, they are equivalent. Therefore, there may be a great need to pay more attention to intermuscular fat deposition in female patients with liver cirrhosis when performing body composition assessment.

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AUTHOR DISCLOSURES

The authors declare no conflict of interest.

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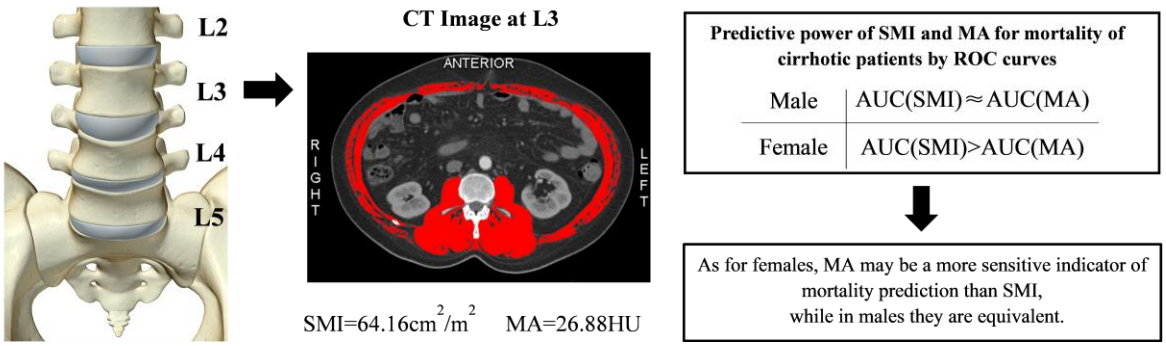

Graphical abstract. SMI: skeletal muscle index; MA: muscle attenuation; HU: Hounsfield units; AUC: area under the curve.

Original Article

Diaphragm thickness on computed tomography for nutritional assessment and hospital stay prediction in critical COVID-19

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Background and Objectives: To evaluate the significance of diaphragm thickness (DT) in assessing the nutritional status and predicting the length of hospital stay (LOS) of patients with COVID-19. **Methods and Study Design:** The data of 212 patients with severe and critical COVID-19 in Wuhan, China, were retrospectively analyzed. Computed tomography (CT)-obtained DT was measured in cross-sectional images of the mediastinal window at the level of the outlet of the celiac trunk at admission and at 2 weeks, then the rate of change in DT(RCDT) at 2 weeks was calculated. Nutritional risk and malnutrition were evaluated at admission. **Results:** A total of 91 patients were involved in the study. The mean DT was 3.06±0.58 mm (3.15±0.63 mm in male and 2.93±0.50 mm in female). DT was significantly negatively correlated with malnutrition based on Global Leadership Initiative on Malnutrition (GLIM) criteria (r=−0.324, p=0.002), Nutritional Risk Screening 2002 (NRS-2002) score (r=−0.364, p=0.000) and the Malnutrition Universal Screening Tool (MUST) score (r=−0.326, p=0.002) at admission. For the prediction of LOS ≥4 weeks in patients with COVID-19, the area under the ROC curve (AUC) of the RCDT at 2 weeks was 0.772, while the AUCs of DT, NRS-2002, MUST and Nutrition Risk in Critically Ill scores at admission were 0.751, 0.676, 0.638 and 0.699 respectively. According to the model of multiple linear regression analysis, the DT at admission (β=−0.377, p=0.000), RCDT at 2 weeks (β =−0.323, p=0.001), and mechanical ventilation (β=0.192, p=0.031) were independent risk factors contributed to LOS. **Conclusions:** CT-obtained DT can be used as a dynamic assessment tool for evaluating the nutritional status of patients in isolation wards for COVID-19.

Key Words: coronavirus disease 2019, nutritional screening, skeletal muscle, diaphragm thickness, length of hospital stay

INTRODUCTION

The global coronavirus disease (COVID-19) epidemic has been present for longer than a year, posing a serious threat to human health worldwide. As of Nov 15, 2021, more than 250 million people have been reported to be infected and more than 5 million people have died.[1] Studies have reported that the prevalence of malnutrition is about 40% of COVID-19 pneumonia patients,[2] while a higher nutritional risk was observed in 61% of the severe COVID-19 pneumonia patients in ICU.[3] Nutritional assessment and support are indispensable components of the treatment regimen for COVID-19. Although many researchers have proposed that nutritional assessment and support in the context of COVID-19 are of great significance,[4,5] there are few studies on the topic. Several traditional nutritional risk scoring tools, such as the Nutritional Risk Screening (NRS), Malnutrition Universal Screening Tool (MUST), and Nutrition Risk in the Critically Ill (NUTRIC) scores, have been used for screening nutritional risks in COVID-19 pneumonia patients.[3,6,7] Considering that COVID-19 usually has an acute onset and rapid progress, these tools have certain limitations and are unable to accurately monitor the nutritional status of the patient throughout the course of the disease. Challenging periods like the COVID-19 pandemic require fast and efficient adaptations of the healthcare system.[8] Therefore, we are interested in determining a novel convenient objective indicator that can accurately and dynamically evaluate the nutritional status of patients with COVID-19.

Skeletal muscle atrophy is an important manifestation of malnutrition and is closely related to the prolonged duration of mechanical ventilation, ICU stay and hospital

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stay, and the increased 1-year mortality.[9] The dynamic monitoring of skeletal muscle indicators, that has received widespread attention, allows for the assessment of nutritional status in real time and can guide nutrition therapy.[10] The diaphragm is a skeletal muscle closely related to respiratory function. More than 60–80% of the tidal volume in spontaneous breathing is produced by diaphragm contraction, which declines under severe disease conditions.[11,12] Diaphragm atrophy has a considerable impact on the prognosis of patients with pneumonia.[13] Therefore, monitoring changes in the diaphragm thickness (DT) may be of clinical significance for the dynamic assessment of nutritional status and guidance of nutritional therapy in patients with COVID-19. We hypothesize that DT is a useful nutritional assessment parameter in severe COVID-19. Bedside ultrasound was recommended to measure respiratory muscles in COVID-19 patients,[14] nevertheless it increased the working hours of medical staff in isolation wards and consumed more personal protective equipment. DT can also be obtained remotely from completed chest computed tomography (CT), which is an important diagnostic method for COVID-19 pneumonia. However, no data to date is available about changes in DT of patients with COVID-19 on CT.

We simplified the protocol for DT measured from CT images,[15] and made the data to be obtained more quickly and clinic-friendly. The objectives of this study were to observe the dynamic changes in the CT-obtained DT of patients with COVID-19 along the course of the disease, evaluate the consistency between nutritional screening tools and assessing DT, and assess the correlation between the change in DT and the length of hospital stay (LOS) of patients with severe COVID-19.

METHODS

Study population

We retrospectively analyzed patients with a confirmed diagnosis of severe and critical COVID-19 who were admitted to three wards at Wuhan No. 1 Hospital and the Guanggu Branch of Tongji Hospital, Wuhan City, Hubei Province, China, supported by two medical teams from February 9 to March 31, 2020. The COVID-19 diagnostic criteria were as follows: 1) a history of residence in an epidemic area, or a history of contact with individuals infected with COVID-19; 2) fever and/or respiratory symptoms; 3) imaging revealing multiple small patchy shadows, interstitial changes, ground-glass opacities, infiltration shadows, and consolidation in both lungs; 4) a normal or decreased total white blood cell count(WBC) and a normal or decreased lymphocyte count in the early stage of the disease; 5) a positive real-time fluorescent reverse transcription polymerase chain reaction test for COVID-19 nucleic acid on nasopharyngeal swab; and 6) severe COVID-19, which was assessed using the following criteria: a) shortness of breath with a respiratory rate ≥30 bpm; b) a resting-state finger pulse oxygen saturation ≤93%; and c) an arterial partial pressure of oxygen or inhaled oxygen concentration ≤300 mmHg. Patients who met one of the following criteria were diagnosed with critical COVID-19: a) respiratory failure occurred and mechanical ventilation was required; b) shock occurred; or c) complicated failure of organs other than the lungs occurred, which required intensive care unit monitoring and treatment. The inclusion criteria for participation in this study were an age ≥18 years and a diagnosis of severe or critical COVID-19. Patients who refused to participate in the study, those with a hospital length of stay of < 2 weeks, and those with missing study data were excluded.

The protocol of this study has passed the review of the ethics committee of NanJing Drum Tower Hospital, the affiliated Hospital of Nanjing University Medical School. (NO.2020-012)

Assessments

The Acute Physiology and Chronic Health Evaluation II (APACHE II), the Sequential Organ Failure Assessment (SOFA), NRS-2002 score, MUST score, NUTRIC score, number of comorbidities, white blood cell, lymphocyte, hemoglobin, prealbumin, albumin and C-reactive protein (CRP) were collected at admission. The patients were diagnosed with malnutrition based on Global Leadership Initiative on Malnutrition (GLIM) criteria at admission. To measure DT based on CT imaging, Image J software was used. The CT cross-sectional images were evaluated in the mediastinal window at the level of the outlet of the celiac trunk, and the intersection between the horizontal tangent line of the anterior edge of the spinal canal and the lateral margins of the diaphragm was selected as the measuring point. The DT was measured vertically to the surface of the diaphragm (Figure 1); each measurement of

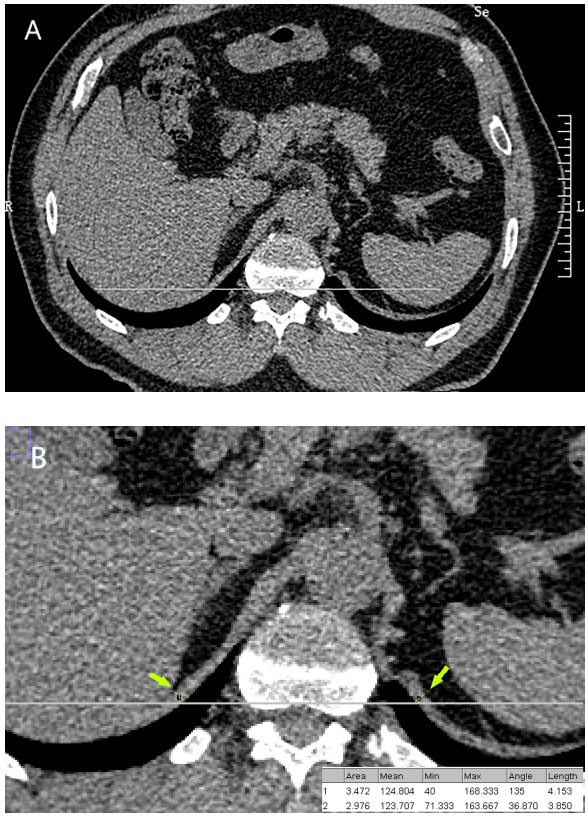

Figure 1. Measurement of DT on chest CT. (A) The CT cross-sectional images were evaluated in the mediastinal window at the level of the outlet of the celiac trunk, and the intersection between the horizontal tangent line of the anterior edge of the spinal canal and the lateral margins of the diaphragm was selected as the measuring point. (B) Image J software was used to measure DT based on CT imaging. The DT was measured vertically to the surface of the diaphragm.

the thickness on the left and right sides of the diaphragm was recorded independently by 3 doctors, and the mean DT of the left and right sides measured 3 times was calculated. The rate of change in DT (RCDT) at 2 weeks was calculated using the following equation:

RCDT at 2 weeks = [(diaphragm thickness at 2 weeks – diaphragm thickness at admission) / diaphragm thickness at admission]

Nutritional support programmes

The attending physicians in isolation wards assessed the nutritional risk and status for each individual at admission, and then set nutrition prescriptions that were online reviewed by dietitians within 24 hours after admission. Oral diet is proposed as the first therapeutic option, and oral enteral nutrition is replenished to achieve nutritional goals. When there is a decrease in oral intake, enteral nutrition will be supplemented by tube feeding. Parenteral nutrition is supplemented in the case of enteral nutrition intolerance over 3 days, from partial dose to full dose as appropriate.

Discharge criteria

The patient discharge criteria were as follows: 1) improved clinical manifestations; 2) chest CT infiltrates were absorbed when compared to the previous examination; 3) two consecutive nucleic acid test results, with an interval of 24 hours, were negative; and 4) the blood lymphocyte count was within the normal range. Patients were divided into two groups based on whether the LOS was ≥4 weeks. A prolonged hospitalization was defined as LOS ≥4 weeks.

Statistical analyses

Normally distributed data are represented as mean ± standard deviation and non-normally distributed data as median. Data on patient characteristics were compared using the χ^2 test and measurement data using analysis of variance and the t-test. Spearman's correlation analysis was conducted to assess the relationship between DT at admission, RCDT at 2 weeks, and the NRS-2002 score, MUST score and NUTRIC score. Receiver operating characteristic (ROC) curves were performed to evaluate the value of DT at admission, RCDT at 2 weeks, and the NRS-2002 score, MUST score, and NUTRIC score at admission in predicting prolonged hospitalization. Moreover, the effect of several variables on LOS was considered with multiple linear regression analysis. The data were processed using statistical software SPSS 22.0 and a two-tailed $p<0.05$ was considered to indicate a statistically significant difference.

RESULTS

Patient baseline data

A total of 212 COVID-19 patients were retrospectively reviewed; however, 65 cases with mild symptoms at admission, 18 cases with unclear CT images of the diaphragm or celiac trunk, and 38 cases with incomplete data (include 2 cases transferred to ICU and 3 cases died within 2 weeks) were excluded (Figure 2). Finally, 91 patients with severe or critical COVID-19 pneumonia were involved in this study. Mean age was 60.5±15.9 years, 52 patients (57.1%) were male, mean body mass index (BMI) was 21.9±2.4, and 17 patients (18.9%) were found to have undernutrition. At admission, the median scores of APACHE II and SOFA were 10 and 2 respectively, the mean DT was 3.06±0.58 mm in total patients (3.15±0.63 mm in male and 2.93±0.50 mm in female), and the mean LOS was 29.1±7.7 days.

There were no deaths in the enrolled patients. 53 patients (58.2%) with NRS-2002 score ≥3 at admission, were divided into the group with nutritional risk, while 38 patients with NRS-2002 score <3 at admission, were divided into groups without nutritional risk. There were significant differences in age, APACHE II score, SOFA score, number of comorbidities, WBC, lymphocyte, hemoglobin, albumin, prealbumin, DT at admission, and LOS between the two groups (Table 1).

Correlation between DT and nutritional risk and status

DT at admission was significantly negatively correlated with malnutrition based on GLIM criteria (r=−0.324, p=0.002), NRS-2002 (r=−0.364, p=0.000) and MUST (r=−0.326, p=0.002) at admission. There was no relativity between DT and NUTRIC score, WBC, lymphocyte, prealbumin, albumin, CRP (Table 2).

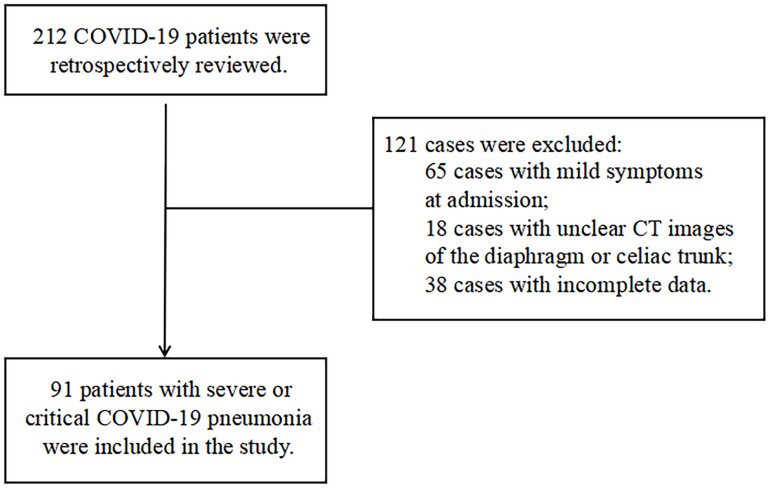

Figure 2. Enrolment of study cases.

Table 1. Baseline clinical and biochemical characteristics of 91 COVID-19 patients

Characteristics	Total (n=91)	NRS-2002 ≥3 (n=53)	NRS-2002 <3 (n=38)	*p*
Age, years	60.5±15.9	65.5±15.7	53.6±13.5	<0.001
Gender				
Male, n (%)	52 (57.1)	33 (62.3)	19(50.0%)	0.244
BMI, kg/m^2	22.3±2.6	21.9±2.78	22.7±2.37	0.157
APACHE II	10 (8-15)	12 (9-18)	8 (5-10)	<0.001
SOFA	2 (2.0-3.0)	3 (2.0-3.5)	2 (2.0-2.0)	<0.001
Number of comorbidities, n (%)				<0.001
0	41 (45.1)	18 (34.0)	23(60.5%)	
1	26 (28.6)	17 (32.1)	9(23.7%)	
≥2	24 (26.4)	18 (34.0)	6(15.8%)	
WBC, ×10^9/L	5.05±2.65	4.23±2.31	6.20±2.68	<0.001
Lymphocyte, ×10^9/L	1.20±0.59	0.99±.036	1.49±0.72	<0.001
Hemoglobin, g/L	123.7±16.5	120.5±17.6	128.2±14.0	0.026
Prealbumin, mg/L	200.8±87.5	181.1±90.4	228.2±76.2	0.011
Albumin, g/L	33.5±4.7	31.6±4.1	36.1±4.4	0.000
CRP, mg/L	81.3±46.3	84.2±47.8	77.3±44.5	0.481
NRS-2002	2.92±1.52	3.96±1.02	1.47±0.69	<0.001
MUST	2.32±0.94	2.83±0.73	1.61±0.72	<0.001
NUTRIC	2.14±1.47	2.88±1.40	1.11±0.80	<0.001
DT at admission, mm	3.06±0.58	2.90±0.52	3.27±0.60	0.002
Male, mm	3.15±0.63	2.99±0.59	3.43±0.60	0.012
Female, mm	2.93±0.50	2.76±0.37	3.11±0.56	0.024
Corticosteroid therapy, n (%)	8 (8.8)	7 (13.2)	1 (2.6)	0.079
Mechanical ventilation, n (%)	7 (7.7)	5 (9.4)	2 (5.3)	0.112
LOS, days	29.1±7.7	31.9±6.8	25.1±7.2	<0.001

APACHE II: acute physiology and chronic health evaluation II; SOFA: sequential organ failure assessment; WBC: while blood cell; CRP: C-reactive protein; NRS-2002: nutritional risk screening 2002; MUST: malnutrition universal screening tool; NUTRIC: nutrition risk in critically ill; DT: diaphragm thickness; LOS: length of hospital stay.

Table 2. Spearman correlation analysis for DT at admission

	Total		Male		Female	
	r	*p*	r	*p*	r	*p*
GLIM	−0.324	0.002	−0.303	0.029	−0.347	0.030
NRS-2002	−0.364	<0.001	−0.426	0.002	−0.375	0.019
MUST	−0.326	0.002	−0.371	0.007	−0.326	0.043
NUTRIC	−0.164	0.119	−0.214	0.128	−0.183	0.265
WBC	0.106	0.318	0.045	0.752	0.267	0.101
Lymphocyte	0.155	0.143	0.040	0.778	0.391	0.014
Prealbumin	0.105	0.320	0.090	0.525	0.166	0.313
Albumin	0.223	0.034	0.322	0.020	0.063	0.704
CRP	0.171	0.105	0.249	0.075	0.071	0.668

DT: diaphragm thickness; r: correlation coefficient; GLIM: Diagnosis of malnutrition based on Global Leadership Initiative on Malnutrition criteria; NRS-2002: nutritional risk screening 2002; MUST: malnutrition universal screening tool; NUTRIC: nutrition risk in critically ill; WBC: while blood cell; CRP, C-reactive protein.

Clinical and nutritional parameters affecting LOS

The patients were divided into two groups according to LOS: ≥4 weeks and <4 weeks. 53 patients (58.2%) were hospitalized for more than 4 weeks, with an average DT at admission of 2.83±0.48 mm, an average DT at 2 weeks of 2.65±0.41 mm, and an average RCDT at 2 weeks of −5.0±15.1%, which were significantly increased in those who were hospitalized for less than 4 weeks, 3.37±0.57 mm, 3.56±0.54 mm, and 6.2±9.7% respectively. 14 patients (26.4%) were diagnosed with malnutrition based on GLIM criteria in the prolonged LOS group, while only 3 patients (7.9%) in the LOS <4weeks group. There were also significant differences in age, SOFA, APACHE II, NRS-2002 score, MUST score, and NUTRIC score at admission between the two groups (Table 3).

ROC Curves in predicting prolonged hospitalization

For the prediction of LOS ≥4 weeks in patients with COVID-19, the area under the ROC curve for the RCDT at 2 weeks was 0.772, for DT at admission was 0.751, for the NRS-2002 was 0.676, for the MUST was 0.638, and for the NUTRIC score was 0.699 (Table 4).

Multivariate linear regression analysis of LOS

LOS was significantly correlated with age (r=0.382, *p*=0.000), APACHE II (r=0.434, p=0.000), number of Comorbidities ≥2 (r=0.222, *p*=0.035), NRS-2002score ≥3 (r=0.435, *p*=0.000), and mechanical ventilation (r=0.306, p=0.003), while LOS was significantly negatively correlated with DT at admission (r=−0.339, *p*=0.001), RCDT at 2 weeks (r=−0.480, *p*=0.000), lymphocyte at admission (r=−0.464, *p*=0.000), prealbumin at admission (r=−0.459, *p*=0.000). Multiple regression analysis showed DT at

Table 3. Clinical and nutritional parameters affecting LOS

	LOS ≥4 weeks (n=53)	LOS <4 weeks (n=38)	*p*
Age, years	65.2±15.6	54.1±14.0	0.001
Gender			
Males, n (%)	30 (56.6)	22 (57.9)	0.902
BMI, kg/m^2	21.8±2.8	22.7±2.3	0.129
SOFA at admission	3.0±1.5	2.3±1.0	0.011
APACHE II at admission	12.8±6.2	9.9±5.2	0.023
GLIM at admission, n (%)	14 (26.4)	3 (7.9)	0.030
NRS-2002 at admission	3.3±1.4	2.4±1.6	0.004
MUST at admission	2.5±0.9	2.0±1.0	0.011
NUTRIC at admission	2.6±1.5	1.6±1.2	0.001
DT at admission, mm	2.83±0.48	3.37±0.57	<0.001
Male, mm	2.88±0.51	3.52±0.58	<0.001
Female, mm	2.77±0.45	3.16±0.49	0.015
DT at 2 weeks, mm	2.65±0.41	3.56±0.54	<0.001
Male, mm	2.67±0.37	3.69±0.63	<0.001
Female, mm	2.63±0.47	3.38±0.35	<0.001
RCDT at 2 weeks, %	-5.0±15.1	6.2±9.7	<0.001

LOS: length of hospital stay; SOFA, sequential organ failure assessment; APACHE II, acute physiology and chronic health evaluation II; GLIM, Diagnosis of malnutrition based on Global Leadership Initiative on Malnutrition criteria; NRS-2002, nutritional risk screening 2002; MUST, malnutrition universal screening tool; NUTRIC, nutrition risk in critically ill; DT, diaphragm thickness; RCDT, the rate of change in diaphragm thickness.

Table 4. ROC curves in predicting prolonged hospitalization

	Sensitivity	Specificity	AUC (95% CI)	*p*
DT at admission	0.553	0.868	0.751 (0.649-0.854)	<0.001
RCDT at 2 weeks	0.895	0.698	0.772 (0.673-0.871)	<0.001
NRS-2002 at admission	0.605	0.717	0.676 (0.560-0.792)	0.004
MUST at admission	0.711	0.472	0.638 (0.522-0.754)	0.025
NUTRIC at admission	0.605	0.717	0.699 (0.591-0.807)	0.001

ROC: receiver operating characteristics; AUC: area under the curve; CI: confidence interval; DT: diaphragm thickness; RCDT: the rate of change in diaphragm thickness; NRS-2002: Nutritional Risk Screening 2002; MUST: malnutrition universal screening tool; NUTRIC: nutrition risk in critically ill.

Table 5. Multivariate linear regression analysis of LOS

	Spearman correlation analysis		Enter regression model			
	r	*p*	B	β	95% CI of B	*p*
Age	0.382	<0.001	0.041	0.101	−0.045-0.128	0.342
Gender	0.052	0.624	---	---	---	---
BMI	−0.201	0.056	---	---	---	---
APACHE II	0.434	<0.001	0.131	0.101	−0.163-0.426	0.387
Comorbidities ≥2	0.222	0.035	0.206	0.012	−2.756-3.169	0.890
NRS-2002 ≥3	0.435	<0.001	0.887	0.057	−2.123-3.898	0.559
DT at admission	−0.339	0.001	−5.008	−0.377	−7.432-(−2.585)	<0.001
RCDT at 2 weeks	−0.480	<0.001	−17.574	−0.323	−28.113-(−7.035)	0.001
Lymphocyte at admission	−0.464	<0.001	−1.531	−0.117	−4.271-1.209	0.270
Prealbumin at admission	−0.459	<0.001	−8.735	−0.099	−24.334-6.864	0.268
CRP at admission	0.143	0.178	---	---	---	---
Mechanical ventilation	0.306	0.003	5.524	0.192	0.523-10.524	0.031
Corticosteroid therapy	0.123	0.246	---	---	---	---

LOS: length of hospital stay; r: correlation coefficient; B: Partial regression coefficient; β: Standardized β; CI: confidence interval; APACHE II: acute physiology and chronic health evaluation II; NRS: nutritional risk screening; DT: diaphragm thickness; RCDT: the rate of change in diaphragm thickness; CRP, C-reactive protein.

admission, RCDT at 2 weeks and mechanical ventilation had a significant influence on LOS (Table 5).

DISCUSSION

The appropriate nutritional monitoring method for patients in infectious isolation wards is still unknown. It is the first study to evaluate the relationship between CT-obtained DT and nutritional risk and nutritional status in COVID-19 pneumonia isolation wards. Our study indicated that CT-obtained DT was significantly correlated with the patient's nutritional risk, nutrition status and LOS, changes in DT occurred dynamically during the course of the disease in patients with COVID-19, and the RCDT at 2 weeks predicted the prolonged hospitalization, after excluding the influence of mechanical ventilation. It suggested that CT-obtained DT might reflect whole-body

muscle mass and the dynamic changes of CT-obtained DT could be a favorable nutritional status assessment tool for rapidly increasing number of patients in isolation wards for COVID-19 pneumonia in this pandemic or potential acute viral pneumonia in the future.

Up to present, symptomatic support is still regarded as an important treatment for COVID-19, especially nutritional therapy, while the body's immune system recovers and regains its defense activity, so as to achieve elimination of the virus.[16] However, patients with COVID-19 often experience different degrees of gastrointestinal symptoms, inflammatory reactions and oxidative stress, resulting in reduced nutrient intake and synthesis, increased catabolism, a high nutritional risk,[17,18] myasthenia and muscular atrophy,[19,20] a prolonged hospital stay, and a high mortality rate for patients with severe disease.[21] More importantly, malnutrition may play a critical role in promoting the transition from mild pneumonia to severe pneumonia.[2,22] Therefore, timely nutritional assessment and support are crucial when managing COVID-19. Nevertheless, due to the emergence of a multitude of cases, including numerous cases of severe disease in a short period of time, medical workers and hospitals have been placed under extreme pressure and the assessment of nutritional status may have been delayed or neglected.

Nutritional risk screening tools such as NRS-2002, MUST, NUTRIC, are widely used to assess the nutritional risk of patients with COVID-19.[3,6,7] However, these tools assess the nutritional risk of the patient, not the nutritional status, and do not evaluate skeletal muscle atrophy. There are obvious shortcomings in using nutritional risk screening tools to monitor dynamic nutritional status during hospitalization. More than half of patients with sarcopenia and myosteatosis are assessed to be at low nutritional risk.[23] Traditional nutritional assessment parameters, such as prealbumin, transferrin, lymphocyte count, triceps brachii skin fold thickness, and grip strength, are susceptible to volume status and inflammatory response; therefore, they have a low predictive value and are unreliable for monitoring a patient's nutritional status.[24] CT is considered the gold standard for the evaluation of total skeletal muscle quantity.[25] In critically ill patients, the psoas muscle index has proven to be a feasible solution to assess the nutritional status.[26] However, this requires an additional CT scan of the psoas major muscle for patients with COVID-19, which increases the burden of epidemic protection and the cost of treatment. Similarly, ultrasound measurement of limb muscles is an attractive option for diagnosing skeletal muscle atrophy and predicting prognosis,[27] although there are many factors, such as excessive compression by the ultrasound probe, obesity, subcutaneous edema, the direction of the probe, and the position of the muscle, that affect the accuracy and reproducibility of the measurement results.[28]

Since a chest CT examination is recommended for the diagnosis of patients with COVID-19, the DT measurement can be obtained in the mediastinal window of the chest CT image; thus, there is no need to increase the scanning scope. For patients with COVID-19, this is an indicator simple to obtain. In this study we detected that diaphragm atrophy at two weeks existed in some patients with severe COVID-19, and it substantially affected the LOS. GLIM, nutritional risk screening scores and DT had a congruent trend. For the prediction of LOS, the measurement of DT and the dynamic changes in DT were superior to the use of nutritional risk screening tools. It is a nutritional assessment index worthy of further research and evaluation in COVID-19 isolation wards.

We believe that CT-obtained DT measurements as a tool for assessing nutritional status has several possible advantages. 1) Considering that the diaphragm is a kind of skeletal muscles, early and continuous high catabolism together with the subsequent skeletal muscle atrophy will affect the diaphragm in critical ill.[29] In addition, once the primary disease is treated, anabolism increases and the diaphragm will show growth similar to the muscles of the limbs and the psoas major muscle, thus reflecting the patient's nutritional status in real time. 2) The diaphragm is the most important inhalation muscle, providing more than 60–80% of the momentum required for inhalation.[30] Diaphragm atrophy may have a more direct impact on respiratory function and prognosis as compared to the limb and psoas major muscles. Therefore, the diaphragm as a nutritional assessment parameter may have a greater clinical significance. 3) Apart from some cases where disuse atrophy of the diaphragm may occur, such as with controlled mechanical ventilation, neuromuscular Junction disease, or diaphragm trauma, most patients maintain normal or even enhanced movement of the diaphragm. Diaphragm atrophy is attributed to increased catabolism of the patient's skeletal muscles under the condition of sepsis, trauma and systemic inflammation; therefore, CT-obtained DT can be more accurate for monitoring nutritional status. 4) As a non bedside nutrition assessment tool, the DT measurements can be obtained using completed chest CT images, no additional examination would be required, and rapid results can be obtained in most cases, without adding additional burden to the already overloaded clinical program and excessive medical expenses, which are feasible factors for promotion in light of the current COVID-19 epidemic.

This study had the following limitations. First, it was a retrospective study with a small sample size and, due to the type of isolation wards, patients were screened based on the severity of their disease prior to being enrolled, resulting in a limited number of deaths and patients with severe disease. Clinical studies concerning COVID-19 in these patients would inevitably cause sampling errors. Second, due to establishing different discharge standards than those that are generally used, using LOS as an observation indicator might have affected the credibility of the results. Third, respiratory movement affected the accuracy of DT measurements. Fourth, a reference range for DT at the level of the celiac trunk on CT is still undetermined. Individual characteristics such as gender, race, and height may also affect the results.

Conclusion

In conclusion, this retrospective study revealed the correlation between CT-obtained DT and nutritional risk and status in patients with COVID-19, confirmed the negative predictive significance of LOS of DT at admission and the RCDT at 2 weeks, and demonstrated that decreased DT at admission and RCDT at 2 weeks may independent-

ly contribute to prolonged hospitalization in these patients. Therefore, it's suggested that CT- obtained DT can be used as a dynamic assessment tool for evaluating the nutritional status of patients in isolation wards for COVID-19. More researches on CT-obtained DT as a dynamic nutritional assessment tool can be pursued in patients with other diseases.

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AUTHOR DISCLOSURES

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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Original Article

Nutrition and clinical manifestations of pulmonary tuberculosis: A cross–sectional study in Shandong province, China

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Background and Objectives: The aim is to investigate the association between nutritional status and clinical picture of pulmonary tuberculosis (PTB). **Methods and Study Design:** A total of 613 pulmonary tuberculosis patients in Weifang city, Shandong province, China were included. Clinical and nutritional history, anthropometry, nutritionally relevant indicators including serum total protein and albumin, hemoglobin and lymphocyte count were measured. Adjustments were made for confounders in multivariable logistic models where tuberculosis activity (clinical symptoms and signs, sputum–smear tests or chest computerized tomography (CT)) was the dependent variable. **Results:** Hypoalbuminemia (OR=2.61; 95% CI, 1.69–4.03), anemia (OR=1.62; 95% CI, 1.04–2.51) and lymphocytopenia (OR=1.92; 95% CI, 1.21–3.05) were associated with a higher TB score (a clinical severity measure for pulmonary tuberculosis based on typical signs and symptoms); hypoalbuminemia (OR=1.75; 95% CI, 1.08–2.84) and anemia (OR=1.87; 95% CI, 1.14–3.08) were associated with a positive sputum smear; anemia (OR=3.58; 95% CI, 1.85–6.94) was associated with cavitation in CT. **Conclusions:** Hypoalbuminemia, anemia and lymphocytopenia were positively associated with the severity of clinical manifestation of PTB. Nutritional status may be a marker for the severity of the clinical manifestations of PTB.

Key Words: pulmonary tuberculosis, nutritional status, clinical manifestations, hypoalbuminemia, anemia

INTRODUCTION

Tuberculosis (TB) is a severe airborne contagious respiratory disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*)[1] and is also a chronic wasting disease. According to estimates from the WHO, approximately 10.0 million cases of TB were reported in 2020, causing approximately 1.4 million deaths; China has a considerably high TB burden, with 8.4% of global TB cases occurring in the country.[2]

Nutrition and its supplementation are important for a number of diseases.[3] Epidemiological studies have revealed that malnutrition plays a key role in the occurrence and development of TB.[4] Severely malnourished patients with PTB (body mass index [BMI] <16) are more likely to exhibit dyspnea, night sweats, hemoptysis, and cavitation.[5] Underweight status at baseline is independently associated with relapse risk among patients with TB.[6] Malnutrition may result in impaired immune function,[7] and increased susceptibility to M. tuberculosis.[8] Moreover, M. tuberculosis infection leads to an acute inflammatory host response,[9,10] accelerating protein loss[11] and inhibiting the production of serum albumin.[12] Hypoalbuminemia objectively reflects malnutrition, and the serum albumin concentration is a primary marker for nutritional status. Lymphocyte count, by contrast, is an objective indicator for the presence of inflammation.[13] Protein and energy deficits contribute to anemia, and patients with anemia are more frequently malnourished than are those without anemia.[14]

However, most studies have investigated the link between active PTB and malnutrition using BMI or underweight status as indicators of nutritional status. The associations between nutritional parameters such as serum total protein levels, the presence of hypoalbuminemia, anemia or lymphocytopenia, and typical clinical signs and symptoms such as the positive sputum smears or lung field lesions found in patients with PTB have received less attention. This study was conducted to examine the associations between the biochemical and hematological indicators that reflect nutritional status and the clinical manifestations present among patients with PTB before TB treatment.

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METHODS

Study design and population

This study was approved by the Medical Ethics Committee of Qingdao Municipal Center for Disease Control and Prevention and follows the Declaration of Helsinki. The study was registered in the Chinese Clinical Trial Registry (registration number ChiCTR–OCC–1900022294). We obtained informed consent from each individual participant, and all data have been maintained in strict confidence during the research process.

In this cross–sectional study, 613 patients with active PTB were selected from local TB clinics in Weifang, Shandong Province, from 2019 to 2021.

The adjusted eligibility criteria were as follows: (1) Patients were newly diagnosed as having PTB (according to China's National Tuberculosis Prevention and Control Guidelines;[15] if sputum smear results were positive, patients were diagnosed as having smear–positive PTB; if sputum specimens were negative and the results of computerized tomography (CT) scans of the chest and the presence of clinical symptoms were compatible with a diagnosis of active PTB, patients were diagnosed as having PTB after discussion with radiologists and clinicians.[15] (2) Participants were ≥18 years old, (3) were free of mental illness, and (4) agreed to sign the informed consent form.

The exclusion criteria were as follows: (1) patients with extrapulmonary tuberculosis, with multidrug–resistant tuberculosis (MDR–TB), or with other pulmonary diseases; (2) patients with severe organ dysfunction or complications such as those related to cardiovascular or lung disease, cancer, or HIV; (3) patients with impaired cognitive function or those with mental illnesses; (4) patients with liver or kidney dysfunction at baseline; or (5) patients who were pregnant or breast–feeding.

Procedures

A standard questionnaire to collect demographic characteristics (including age, gender, education level, area of residence, marital status, smoking and drinking) and clinical manifestations was administered by trained staff. The initial clinical manifestations of patients with PTB were assessed using a standard questionnaire to calculate a TB score. The TB score was calculated according to modified previous methods[16-18] and was used as a comprehensive index for the assessment of initial clinical symptoms. The TB score was based on the presence of typical manifestations of active PTB, including cough, sputum production, hemoptysis, chest pain, fever, night sweats, fatigue and loss of appetite. Patients who reported any of the eight listed symptoms, scored one point for each symptom. We calculated patient BMI through the following formula: BMI = weight (kg) / height (m)2. For a BMI of <16, 16–18, >18, two, one, and zero points were added to the TB score, respectively. In total, the TB scores ranged from 0 to 10. The patients were divided into two groups according to their TB score: patients with ≤3 points and patients with >3 points.

Sputum specimens were examined by microscopy to determine the presence and number of acid–fast bacilli (AFB). The AFB smears were examined through Ziehl–Nielsen acid–fast staining and the results were categorized as either positive or negative for the presence of AFB indicating active PTB. CT scans were conducted through the spiral technique, with the results of the CT scans categorized as exhibiting cavitation or not.

Peripheral venous blood was collected from each patient after an overnight 8–12–h fasting period. Blood was drawn from the antecubital vein using aseptic venipuncture from the cubital fossa and collected into labelled plain test tubes. The 5–mL blood sample was allowed to clot and subsequently centrifuged at 4000 rpm for 10 minutes. An automatic biochemical analyzer (Beckman AU5800) and an automatic hematology analyzer (XN1000) were employed to assay total protein (TP), albumin (ALB), hemoglobin (Hb) and lymphocyte count.

The reference value range for normal values was designated as TP: 60–80g/L.[19] Hypoalbuminemia was defined as an ALB value <35 g/L.[20] Anemia was determined according to Hb concentration (for men <120 g/L, for women <110 g/L).[21] Lymphocytopenia was defined as a lymphocyte count <1.0 (10^9/L).[22]

The investigators were trained by project members who checked the completed questionnaires. The data were entered independently by two project members and were verified twice.

Statistical analyses

The data analyses were conducted using SPSS 23.0. Quantitative data that conformed to a normal distribution are expressed as means and (standard deviations). Quantitative data that were not normally distributed are reported as medians and interquartile ranges. The distribution of categorical variables such as gender, education level, area of residence, marital status, smoking and drinking are presented as frequencies and percentages. The biochemical and hematological indicators related to nutritional status as independent variables were divided into two groups. A binary logistic regression analysis with forward stepwise strategy was performed to measure associations between nutritional parameters and the clinical manifestations of PTB. Normal biochemical and hematological indicators were used as a reference.

In the multivariate analysis, model 1 was adjusted for potential confounding factors that included age, gender, BMI, smoking, drinking, education level, area of residence, and marital status. Adjusted ORs with 95% CI are reported for the results of the logistic regression to indicate the strength and direction of the associations. All tests were two–sided, and $p<0.05$ or $p<0.01$ was considered statistically significant.

RESULTS

This cross–sectional study included 613 patients diagnosed as having PTB (Figure 1). The demographic characteristics are listed in Table 1. Sputum smear tests were conducted for 473 patients, with 208 (44.0%) being sputum-smear positive (Figure 1); moreover, 352 patients were evaluated by CT, with 142 (40.3%) patients exhibiting cavitation (Figure 1). Among the 613 participants, 67.5% were men. The majority of patients were between the ages of 18 and 44 years (58.2%), with the average age being 41.5±18.0 years. Most of the participants (63.6%) were married and were from rural areas (69.7%). The

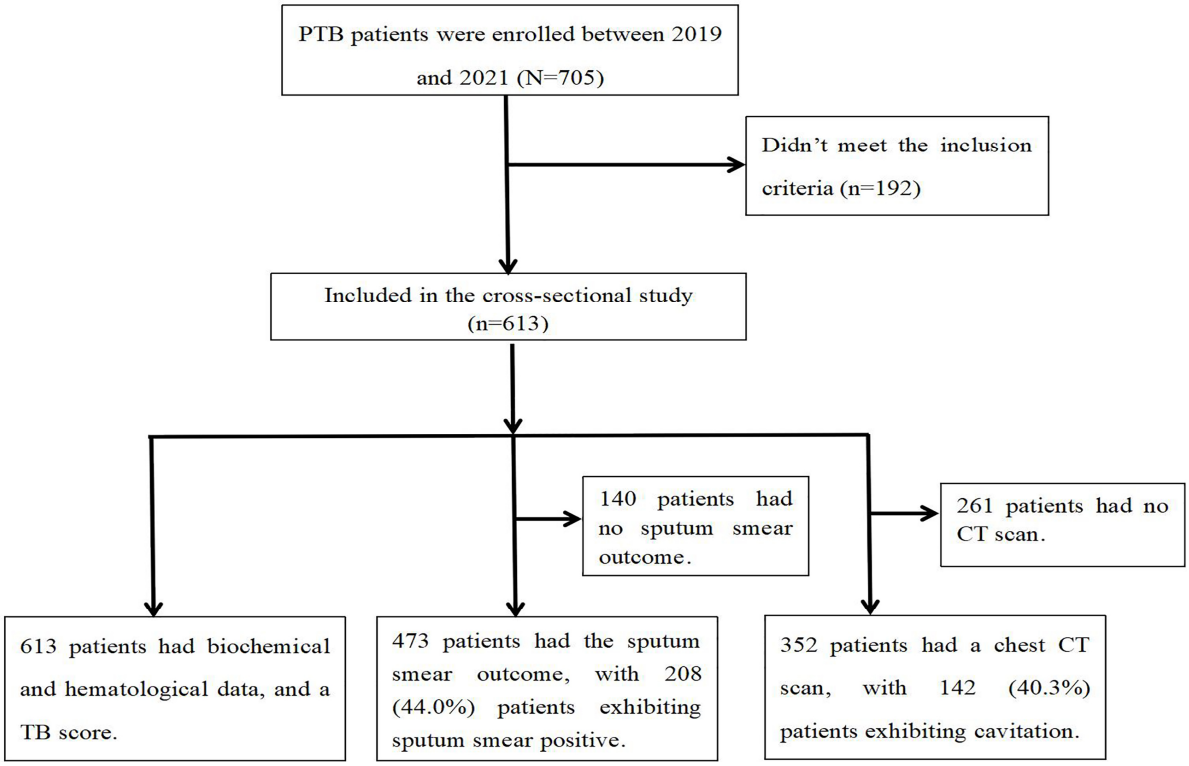

Figure 1. Study flow chart.

mean BMI of patients was 20.7, with 27.6%, 57.3%, and 15.2% of patients being underweight, normal weight, and overweight or obese, respectively. Moreover, 38.7% of the patients consumed alcohol, and 36.7% consumed tobacco.

The prevalence of each contributing parameter in the TB score is listed in Table 2. TB scores were conducted for 613 patients, with 213 (34.7%) exhibiting TB scores >3. The medians (interquartile ranges) of TB scores were 3.0 (2.0, 4.0). The most frequent clinical symptoms of tuberculosis patients were cough (75.0%) and sputum production (56.1%). Fatigue (30.0%) and fever (33.9%) were present in more than one-third of respondents. Hemoptysis (14.2%), chest pain (18.1), and night sweats (9.6%) were less common among patients with PTB.

The mean concentrations of serum TP, ALB, peripheral blood Hb, and lymphocyte counts among the study participants were 68.9±7.49 g/L, 38.7±5.74 g/L, 129.3±18.3g/L, and 1.52±0.64 (10^9/L), respectively. Among the 613 patients with PTB, 153 (25%), 130 (21.2%), and 111 (18.1%) patients exhibited hypoalbuminemia, anemia, and lymphocytopenia (Table 1), respectively.

After adjustments were made for multiple confounding factors, a higher TB score (TB score >3 points group) was positively associated with hypoalbuminemia (OR=2.61; 95% CI, 1.69–4.03), anemia (OR=1.62; 95% CI, 1.04–2.51) and lymphocytopenia (OR=1.92; 95% CI, 1.21–3.05) (Table 3). The associations between nutritional parameters of patients with PTB and typical clinical symptoms of PTB are presented in Table 4. In multivariate logistic regression, model 1 indicated that patients with PTB exhibited TP levels lower than the reference range, exhibited fever more frequently (OR=1.99; 95% CI, 1.12–3.54), and experienced greater loss of appetite (OR=2.21; 95% CI, 1.22–4.02); moreover, patients with PTB with hypoalbuminemia exhibited cough more frequently (OR=4.47; 95% CI, 2.31–8.66), exhibited greater sputum production (OR=1.78; 95% CI, 1.15–2.76) as well as fever (OR=3.98; 95% CI, 2.56–6.19), fatigue (OR=2.00; 95% CI, 1.30–3.09), loss of appetite (OR=1.92; 95% CI, 1.23–3.01) and weight loss (OR=1.81; 95% CI, 1.08–3.03). The presence of anemia among patients with PTB was associated with more frequent coughs (OR=1.99; 95% CI, 1.13–3.50), greater sputum production (OR=1.62; 95% CI, 1.03–2.54), presence of fever (OR=2.40; 95% CI, 1.56–3.68), fatigue (OR=1.81; 95% CI, 1.17–2.82), loss of appetite (OR=1.63; 95% CI, 1.03–2.56) and weight loss (OR=2.00, 95% CI, 1.20–3.33). Patients with PTB with lymphocytopenia experienced more frequent coughs (OR=3.10; 95% CI, 1.57–6.10), fever (OR=2.67, 95% CI, 1.71–4.17), fatigue (OR=1.67; 95% CI, 1.06–2.64), loss of appetite (OR=1.98; 95% CI, 1.23–3.19), and weight loss (OR=1.84; 95% CI, 1.08–3.14). Table 5 summarizes model 1, where multiple potential confounding factors were considered. Compared with the negative sputum–smear group, the results of the positive sputum smears were associated with hypoalbuminemia (OR=1.75; 95% CI, 1.08–2.84) and anemia (OR=1.87; 95% CI, 1.14–3.08). Compared with the non–cavitation group, the presence of cavitation was strongly associated with anemia (OR=3.58; 95% CI, 1.85–6.94; Table 6). Moreover, as is indicated in Table 3, Table 5 and Table 6, the proportions of TB scores >3 points, positive sputum smear test results, and the presence of cavitation in patients combined with TPs below the reference value were 53.2%, 49.0% and 52.0%, respectively. For patients with hypoalbuminemia, the values were 58.8%, 62.3% and 56.5%, respectively, and for patients with anemia, the values were 52.3%, 62.2% and 60.9%, respectively. For patients with lym-

Table 1. Baseline characteristics for the included pulmonary tuberculosis patients

Characteristics	N	%
Age-group		
18-44	357	58.2
45-65	180	29.4
≥66	76	12.4
Gender		
Women	199	32.5
Men	414	67.5
Residence		
Urban	186	30.3
Rural	427	69.7
Marital status		
Single	195	31.8
Married	390	63.6
Widowed	15	2.4
Divorced	13	2.1
Education		
Unknown	17	2.8
illiteracy	24	3.9
Primary and junior high school	246	40.1
Senior and technical secondary school	244	39.8
Diploma or higher	82	13.4
Alcohol consumption		
Yes	237	38.7
No	376	61.3
Smoking status		
Smoker	225	36.7
Non-smoker	388	63.3
BMI (kg/m^2)		
<18.5	169	27.6
18.5-23.9	351	57.3
≥ 24.0	93	15.2
Hypoalbuminemia		
Yes	153	25.0
No	460	75.0
Anemia		
Yes	130	21.2
No	483	78.8
Lymphocytopenia		
Yes	111	18.1
No	502	81.9

BMI: body mass index.
Categorical variables are presented as N and %

Table 2. Clinical symptoms and TB score for the included pulmonary tuberculosis patients†

Parameters	Points assigned	N (%)
Cough	1	460 (75.0)
Sputum production	1	344 (56.1)
Hemoptysis	1	87 (14.2)
Chest pain	1	111 (18.1)
Fatigue	1	184 (30)
Night Sweats	1	59 (9.6)
Fever	1	208 (33.9)
Loss of appetite	1	163 (26.6)
BMI<16	2	35 (5.7)
16≤BMI≤18	1	97 (15.8)
BMI>18	0	481 (78.5)

BMI: body mass index.
†Categorical variables are presented as N and %

phocytopenia, the values for those with TB scores >3 points, positive sputum smear tests, and cavitation were 54.1%, 55.8% and 57.6% respectively.

DISCUSSION

In the present study, we observed that hypoalbuminemia, anemia, and lymphocytopenia were positively associated with the severity of initial symptoms (as indicated by the increased TB scores), with positive sputum smears, and with the presence of cavitation before anti–tuberculosis treatment in patients with PTB. Additionally, abnormal values for biochemical and hematological indicators were strongly related to the typical clinical signs and symptoms of PTB. Therefore, our data suggest that the poor nutritional status may affect the severity of the initial clinical manifestations of PTB.

Malnourished patients with TB have lower serum albumin concentrations.[12] One possible reason is that protein–calorie malnutrition reduces the effectiveness of host defense mechanisms and cell–mediated immunity[23] and also lowers visceral protein levels[24,25] in patients with TB. Our study revealed that hypoalbuminemia was positively associated with the severity of clinical signs and symptoms and positive sputum smear results. Poor nutritional status has been reported as significantly associated with a higher number of symptoms (fever and weight loss), and a higher proportion of cases that were sputum–smear positive.[26] Hypoalbuminemia has been highlighted as a predictive risk factor for in–hospital mortality in patients with TB.[27] By contrast, weight loss and malnutrition in patients with PTB may be caused by a reduction in food intake or other TB–related factors such as metabolic abnormalities, poor nutrient absorption, fever, and anorexia.[4] This may lead to the "anabolic block" that occurs in patients with TB, whereby ingested amino acids are utilized for oxidation rather than for protein synthesis.[28]

Anemia is common among patients with TB and is associated with the inhibition of erythropoietin production caused by the increased presence of cytokines and changes in iron metabolism.[29] In our study, we found that being anemic was significantly associated with more severe clinical presentation as evidenced by a higher TB score among patients with PTB. Moreover, patients who were anemic were more likely to exhibit positive sputum smear results. This finding is similar to that of a survey conducted in Tanzania that determined that anemia was associated with delayed sputum smear conversion.[30] Our study revealed that patients with PTB and anemia also exhibited cavitation on chest CT. Studies have indicated that patients with TB presenting with increased numbers and greater diversity of lung lesions exhibited lower concentrations of hemoglobin, suggesting anemia,[31] and the affected lungs and cavitation were significantly associated with hemoglobin levels.[32] Population studies have reported that malnutrition leads to the downregulation of type 1 cytokines such as interleukin (IL)–2 and interferon-gamma (INF-γ), and to the upregulation of type 2 cytokines such as IL–4 and IL–10.[33] These cytokines can change pathogenesis of TB under the impact of malnutrition on bacterial clearance, and lead to the formation of cavitation.[34] One study demonstrated that larger cavity volume, especially that in closer proximity to the airways, was associated with higher cough frequency, higher bacillary burden, and delayed culture conversion.[35]

Table 3. Logistic regression analysis for the association between nutritional status and TB score at baseline (N=613)

	TB score >3 n (%)	OR (95% CI)	
		Crude model†	Model 1‡
TP below the reference			
No	180 (32.7)	Reference	Reference
Yes	33 (53.2)	2.35 (1.38, 3.98)*	1.68 (0.920, 3.07)
Hypoalbuminemia			
No	123 (26.7)	Reference	Reference
Yes	90 (58.8)	3.91 (2.67, 5.74)**	2.61 (1.69, 4.03)**
Anemia			
No	145 (30.0)	Reference	Reference
Yes	68 (52.3)	2.56 (1.72, 3.77)**	1.62 (1.04, 2.51)*
lymphocytopenia			
No	153 (30.5)	Reference	Reference
Yes	60 (54.1)	2.68 (1.77, 4.08)**	1.92 (1.21, 3.05)**

TP: total protein; N: the total number of patients in TB score>3 group
†Crude model was not adjusted.
‡Model 1 was adjusted for age, gender, smoking, drinking, BMI, education completed, marital status, residence.
*p<0.05, **p<0.01

Table 4. Logistic regression analysis for the association between nutritional status and clinical symptoms of pulmonary tuberculosis

	OR (95% CI) †					
	Cough	Sputum production	Fever	Fatigue	Loss of appetite	Weight loss
TP lower than reference	1.34 (0.631, 2.84)	1.24 (0.677, 2.27)	1.99 (1.12, 3.54)*	1.60 (0.898, 2.86)	2.21 (1.22, 4.02)**	1.52 (0.775, 2.96)
Hypoalbuminemia	4.47 (2.31, 8.66)**	1.78 (1.15, 2.76)*	3.98 (2.56, 6.19)**	2.00 (1.30, 3.09)**	1.92 (1.23, 3.01)**	1.81 (1.08, 3.03) *
Anemia	1.99 (1.13, 3.50)*	1.62 (1.03, 2.54)*	2.40 (1.56, 3.68)**	1.81 (1.17, 2.82)**	1.63 (1.03, 2.56)*	2.00 (1.20, 3.33)**
lymphocytopenia	3.10 (1.57, 6.10)**	1.48 (0.930, 2.36)	2.67 (1.71, 4.17)**	1.67 (1.06, 2.64)*	1.98 (1.23, 3.19)**	1.84 (1.08, 3.14)*

TP: total protein.
†OR (95%CI): odds ratio (95% confidence interval) adjusted for age, gender, smoking, drinking, BMI, education completed, marital status, residence.
*p<0.05, **p<0.01.

Table 5. Logistic regression analysis for the association between nutritional status and sputum smear results (N=473)

	AFB (+), n (%)	OR (95% CI)	
		Crude model†	Model 1‡
TP below the reference			
No	189 (43.4)	Reference	Reference
Yes	25 (49.0)	1.26 (0.702, 2.25)	0.807 (0.428, 1.52)
Hypoalbuminemia			
No	137 (38.2)	Reference	Reference
Yes	71 (62.3)	2.68 (1.73, 4.13)**	1.75 (1.08, 2.84)*
Anemia			
No	147 (39.2)	Reference	Reference
Yes	61 (62.2)	2.56 (1.62, 4.04)**	1.87 (1.14, 3.08)*
lymphocytopenia			
No	160 (41.3)	Reference	Reference
Yes	48 (55.8)	1.79 (1.12, 2.87)*	1.31 (0.786, 2.19)

AFB (+): acid-fast bacilli smear positivity; TP: total protein.
†Crude model was not adjusted.
‡Model 1 was adjusted for age, gender, smoking, drinking, BMI, education completed, marital status, residence.
*p<0.05, **p<0.01.

Our study found a significant association among lymphocytopenia and cough, fever, fatigue, loss of appetite, weight loss, and higher TB scores in patients with PTB. Studies have reported that CD4 lymphocytopenia was associated with positive sputum smear results and signs of cough and wasting.[36] Patients with TB with extended disease frequently exhibit malnourishment and lymphopenia.[37] Previous animal experiments have indicated that nutritional deficiencies resulted in reduced lymphocyte counts, inability to endure the increased production of

Table 6. Logistic regression analysis for the association between nutritional status and lung cavitation (N=352)

	Cavitation, n (%)	OR (95% CI)	
		Crude model†	Model 1‡
TP below the reference			
No	129 (39.4)	Reference	Reference
Yes	13 (52.0)	1.66 (0.736, 3.76)	0.985 (0.395, 2.46)
Hypoalbuminemia			
No	94 (35.2)	Reference	Reference
Yes	48 (56.5)	2.39 (1.45, 3.92)**	1.59 (0.873, 2.88)
Anemia			
No	100 (35.3)	Reference	Reference
Yes	42 (60.9)	2.85 (1.66, 4.89)**	3.58 (1.85, 6.94)**
lymphocytopenia			
No	108 (36.9)	Reference	Reference
Yes	34 (57.6)	2.33 (1.32, 4.11)**	1.73 (0.899, 3.33)

TP: total protein.
†Crude model was not adjusted.
‡Model 1 was adjusted for age, gender, smoking, drinking, BMI, education completed, marital status, residence.
*p<0.05, **p<0.01.

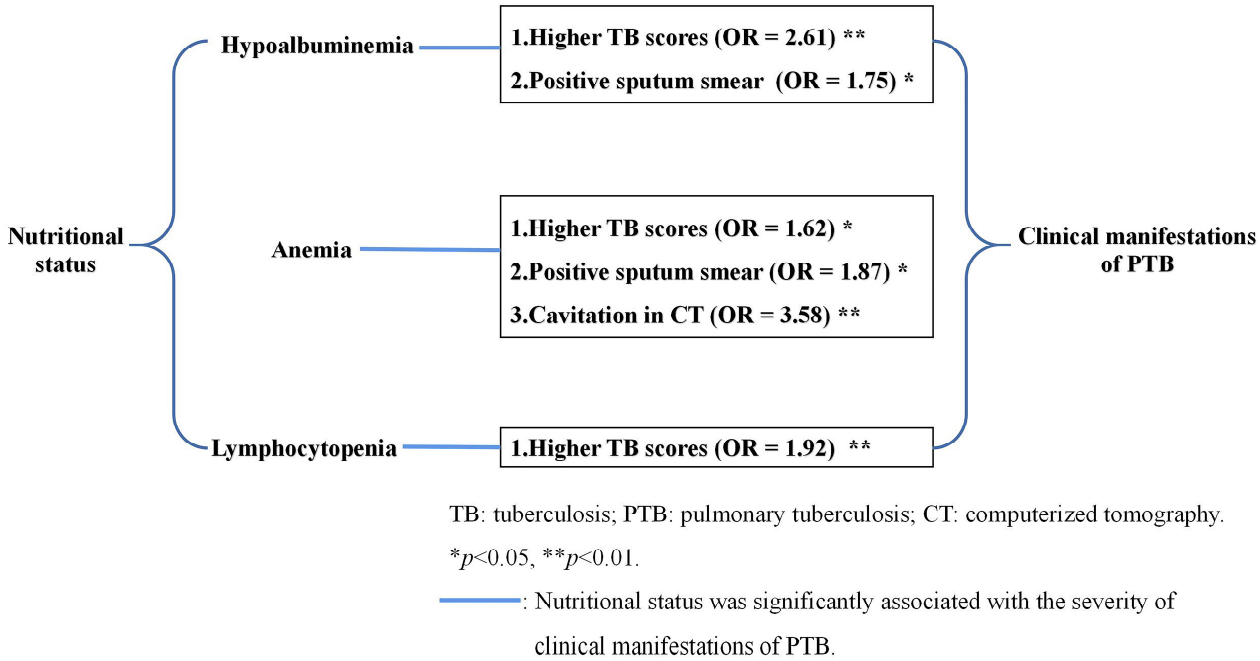

Figure 2. Graphical abstract.

INF–γ[38] and decreased the expressions of nitric oxide synthase (NOS)-2,[39] which plays a crucial role in generating mycobactericidal nitrogen oxide.

The present study has several limitations. First, this was a cross–sectional study that employed a single timepoint assessment, and only included hospitalized patients. Prospective studies are required to confirm our results. Second, the associations between nutritional parameters and the initial clinical manifestations of PTB do not indicate a cause–and–effect relationship at the time of diagnosis. Third, the population of this study did not include any patients with MDR–TB. MDR–TB is known to strongly affect the nutritional status of patients with TB. The present results, therefore, should not be applied to areas where MDR–TB is prevalent.

Despite these limitations, this study has some strengths and merits. First, the TB score is a comprehensive index used to indicate the severity of initial tuberculosis symptoms. Second, hematological and biochemical measurement is a low–cost procedure that is simple to perform and available to tuberculosis patients upon hospital admission. Moreover, some markers can be used to determine patient nutritional status.

Conclusion

The results of this study reveal that poor improvements in nutritional status during the initial phases of PTB treatment are associated with a risk of more severe clinical signs and symptoms, positive sputum smear results, and the presence of cavitation. These results suggest that greater attention should be given to the crucial role that malnutrition plays in patient disease management. Alleviating the severity of PTB may require the synergistic effects of nutritional recovery and TB therapy.

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AUTHOR DISCLOSURES

The authors declare no conflict of interest.

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Original Article

Insulin for hyperglycemia prevention and management during postgastrectomy nutrition support in gastric cancer: Reduced complications in a retrospective cohort study in China

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Background and Objectives: To evaluate the effectiveness of insulin addition to the total nutrition admixture (TNA) for glycemic control among patients with gastric cancer (GC) receiving supplementary parenteral nutrition (SPN) after gastrectomy. **Methods and Study Design:** A retrospective cohort study was conducted among 208 noncritical ill patients who underwent gastrectomy for GC from 2017 to 2019 at a tertiary teaching hospital in Lanzhou, China. All the included patients received individualized SPN and enteral nutrition treatment after gastrectomy. The patients were randomly divided into insulin and noninsulin groups based on the TNA composition. Blood glucose (BG) measurements, glycemic fluctuation, and hypoglycemia incidence during SPN were compared between the two groups. The postoperative comprehensive complications index (CI) and infections were compared according to insulin regimen and postoperative glycemic status. **Results:** The mean BG was significantly lower and fluctuated less in the insulin group than in the noninsulin group ($p<0.05$). One unit of insulin per 6 g of parenteral nutrition glucose addition to TNA did not increase hypoglycemia incidence ($p>0.05$). Comparing CI and the infection rate, no significance was observed between the insulin and noninsulin groups, but a higher postoperative CI was observed in patients with hyperglycemia than in euglycemic patients ($p<0.05$). **Conclusions:** Appropriate insulin addition to TNA has an overall positive effect on glycemic management in patients with noncritical GC who received SPN after gastrectomy. Postoperative glycemic status was associated with the incidence of relevant complications. Further research is needed for conclusive recommendations.

Key Words: gastrectomy, supplementary parenteral nutrition, hyperglycemia, insulin, blood glucose fluctuation

INTRODUCTION

Gastric cancer (GC) is the fifth most common type of tumor and the third leading cause of cancer-related death worldwide.[1] Radical gastrectomy is an effective treatment option for patients with GC. Although the enhanced recovery after surgery concept is commonly promoted perioperatively, many nondiabetic patients present with hyperglycemia (HG) due to the long operation time, large postoperative trauma, nutritional support, and possible anxiety. Poor control of perioperative blood glucose (BG) is closely associated with an increased chance of postoperative complications and mortality in patients who underwent major abdominal surgeries,[2-4] and nondiabetic patients who experience HG have an increased risk of infection.[2]

Insulin therapy is the best method of glycemic control in the hospitalization setting. For non–intensive care unit (ICU) patients who receive parenteral nutrition (PN), direct insulin addition to total nutrition admixture (TNA) has been recommended in some studies,[5-7] as it is a simple and less painful procedure. Nevertheless, according to the Chinese consensus for PN compounding, prophylactic insulin should not be administered to euglycemic patients who receive PN.[8] In practice, however, we observed the increased occurrence of HG and pertinent complications in nondiabetic patients undergoing surgery who did not

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receive insulin therapy while on PN. Regarding this topic, we reviewed relevant studies and found that some recommend 1 unit of insulin per 4–10 g of PN glucose to be routinely added to the TNA for nondiabetic PN patients for glycemic control.[9,10]

Currently, solid evidence of the effects of insulin in TNA on glycemic control is still lacking, particularly among patients with postoperative cancer. To optimize the glycemic management protocol for our patient population, in this retrospective cohort study, we assessed the safety and efficacy of insulin addition to TNA through the analysis of postoperative BG concentrations and the complication incidence among 208 patients with GC who received supplementary parenteral nutrition (SPN) after gastrectomy.

METHODS

Study design and participants

In total, 208 nondiabetic patients with GC received surgical intervention in our surgical oncology department between March 2017 and September 2019. Participant inclusion criteria were as follows: (1) histologically confirmed diagnosis of gastric adenocarcinoma; (2) underwent elective radical gastrectomy and D2 lymphadenectomy; and (3) at nutritional risk, with nutritional risk screening (2002) score ≥3. Patients with the following criteria were excluded: (1) preexisting diabetes or diabetes diagnosis during hospitalization (i.e., admission random venous plasma glucose [VPG] >11.1 mmol/L [200 mg/dL]); (2) coexistence of other malignancy; (3) systemic glucocorticoid treatment within 3 months before admission or during hospitalization; (4) patient directly transferred to ICU after surgery; (5) incomplete postoperative BG data; and (6) HG occurrence after infection onset. Clinical data, including BMI, surgical method, pathological stage, postoperative BGs, and postoperative complications, were evaluated. The study was performed according to the Declaration of Helsinki and was approved by the Ethics Committee of the First Hospital of Lanzhou University (Ethical approval number: LDYYLL-2021-272).

Interventions

All the 208 included patients underwent radical gastrectomy (proximal gastrectomy, distal gastrectomy, or total gastrectomy) with D2 lymphadenectomy. Postoperative pathological staging was performed according to the American Joint Committee on Cancer, eighth edition, staging system.

Patients were allowed to sip water or were provided nasojejunal tube feeding (NJTF) of 5% glucose sodium chloride solution up to 300 mL from postoperative day 1 (POD1). From POD2, patients without gastrointestinal symptoms, including diarrhea, abdominal pain, abdominal distension, and vomiting, were initiated on an oral liquid diet or NJTF (Fresubin), which provided 25% of the estimated total energy expenditure (TEE) calculated using Harris Benedict equation. Feeding was adjusted based on the patient's tolerance and oral intake. All patients received individualized SPN through central venous access from POD1 until the total enteral nutrition reached 60% of the TEE to prevent progressive malnutrition. SPN was formulated daily in accordance with the relevant guidelines[11] to ensure the total nutritional intake meet at least 60% of the TEE. Individualized dosages of vitamins, minerals, and trace elements were added to solutions. All TNAs were timely compounded in the Pharmacy Intravenous Admixture Services center of the hospital.

BG monitoring and evaluation

The BG data of all recruited patients were recorded, including admission random VPG, capillary blood glucose (CBG) after returning to the ward on the operation day (recorded as pre-SPN CBG), POD1 VPG (at 07:00), and during SPN CBG (four times daily at 06:00, 12:00, 18:00, and 00:00).

The collected BG data were statistically evaluated based on the following indictors. Blood glucose control rate (BGCR): the ratio of BG values within 3.9–10 mmol/L (70–180 mg/dL). Hypoglycemia incidence: the number and proportion of hypoglycemia (BG <2.8 mmol/L [50 mg/dL]) in each subgroup. HG incidence: the number and proportion of patients with HG (BG >11.1 mmol/L [200 mg/dL]) more than twice[12] in each subgroup. Coefficient of variation (CV): the ratio of glycemic standard deviation to the mean. Fasting capillary glucose -CV (FCG-CV):[13] the ratio of the standard deviation of fasting CBG (collected daily at 06:00 AM). The largest amplitude of glycemic excursions (LAGE): the difference between the maximum and minimum BG values during SPN.

Postoperative complications

Postoperative comprehensive complications index (CI)[14] within 30 days after surgery was determined. Postoperative infections were graded according to the Clavien-Dindo Classification[15] and included superficial and deep wound infection, organ/space infection, urinary tract infection, pneumonia, sepsis, and septic shock.[16]

Statistical analysis

The experimental data were recorded in a Microsoft Excel (version 2010) spreadsheet and were statistically analyzed and visually processed using SPSS (version 26.0) and GraphPad Prism (version 8.0), respectively. All continuous variables were examined using the normal distribution test. The independent t test (normally distributed, mean ± standard deviation) and Mann–Whitney U test (not normally distributed, median [interquartile range: Q1, Q3]) were performed for between-group comparisons. The chi-square test and Fisher's exact test were used for categorical data analysis (n [%]). Two-tailed p values of <0.05 were considered statistically significant.

RESULTS

In total, 208 patients (158 men and 50 women) with a mean age of 59.4 years were enrolled into this study (Figure 1). The insulin and noninsulin groups consisted of 89 (42.8%) and 119 (57.2%) patients, respectively. We obtained 3254 BG measurements (average of 15.6 measurements per subject) at different time points of assessment from the two groups. The demographic (age, sex, BMI) and clinical characteristics (history of hypertension, neoadjuvant chemotherapy, admission VPG, and patho-

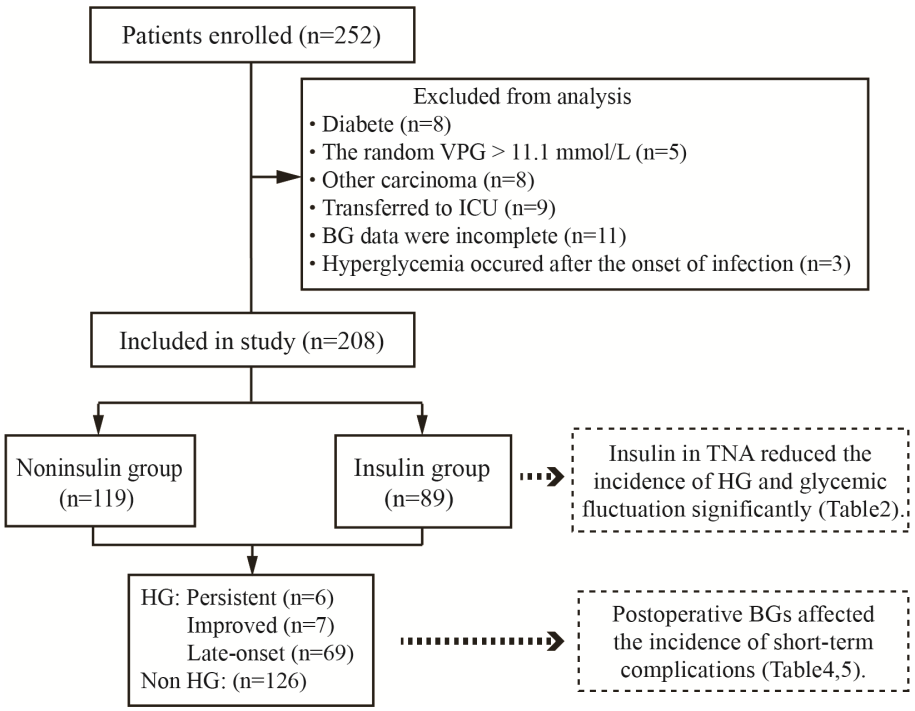

Figure 1. Flowchart of patient selection.

logical tumor–node–metastasis stage) were comparable (p>0.05) between the two groups (Table 1).

Before SPN was initiated, no statistical difference was observed in POD1 VPG and HG incidence between the groups (Table 1). During SPN, the mean BG concentrations and HG incidence in the insulin group were significantly lower than those in the noninsulin group (p<0.05; Table 2). Furthermore, >25% of the patients in the insulin group achieved the BGCR, which was significantly higher than that in the noninsulin group. Adding 1 unit of insulin per 6 g of PN glucose in TNA did not significantly increase the incidence of hypoglycemic events (Table 2). The BG values before and during SPN were analyzed. Patients with pre-SPN HG were categorized into persistent HG (HG appearing before and during SPN) and improved HG (pre-SPN HG resolved while receiving SPN) groups. Patients without pre-SPN HG were categorized into late-onset HG (BG was normal before SPN, and HG developed while on SPN) and non-HG (BG within normal limit before and during SPN) groups. During SPN, we observed numerous patients with late-onset HG in the noninsulin group (46.2% vs 15.7%, p<0.05) (Table 2). Regarding patients with pre-SPN HG, 62.5% (5/8) of the BG measurements in the insulin group normalized during SPN compared with 40% (2/5) in the noninsulin group.

Glycemic fluctuation is a risk factor for increased postoperative complications and mortality.[17] We listed the mean and highest BGs from POD1 to POD3 in Figure 2, and we calculated the amplitude and CV (LAGE, CV, and FCG-CV) to evaluate the glycemic status while patients were on SPN. As shown in the results, the CV and LAGE calculated according to days indicate unstable BGs during SPN, and insulin addition to TNA had a positive effect on glycemic maintenance (Table 2).

Although the incidence of grade III–V infection observed in the noninsulin group was more than that observed in the insulin group, the postoperative CI and infection rates were not statistically different between the groups (Table 3). However, considering the effect of HG, CI (p<0.05) and the incidence of infection were significantly higher in patients with postoperative HG than in euglycemic patients (Table 4). In subgroup analysis, the infection rate and CI were statistically significantly higher in patients with persistent HG, improved HG, and late-onset HG than in euglycemic patients, irrespective of insulin therapy (Table 5). Among the subgroups, the incidence of grade III–V infection was the highest in the late-onset HG group (Table 5). No statistical difference was observed in infection complications, and a few infection cases were caused by surgical complications that were irrelevant to the glycemic status. We observed three infectious cases in the non-HG group that were caused by abdominal bleeding, anastomotic leakage, and adhesive intestinal obstruction; two patients in the late-onset HG group presented with sepsis shock due to pulmonary infection and severe incision suppuration, respectively.

DISCUSSION

Several studies have revealed that HG is closely related to the prognosis of patients postoperatively. This retrospective study investigated the glycemic effects of insulin addition (1 unit/6 g glucose) to TNA among nondiabetic patients with GC who received SPN after elective radical gastrectomy. Our study suggested that the postoperative CI and infection rate were significantly higher in patients with HG than in euglycemic patients, and insulin addition to TNA efficiently reduced the postoperative BG concentrations and fluctuation. Furthermore, this insulin dosage in TNA did not increase hypoglycemia occurrence.

Several studies have shown that HG may impair immune function through the reduction of phagocytic activity of macrophages, chemotaxis disruption of polymorphonuclear neutrophils, increase in adhesion molecule expression, and free radical production in immune cells,

Table 1. Baseline characteristics of participants

	Noninsulin (n=119)	Insulin (n=89)	*p*-value
Sex [n (%)]			0.843†
Men	91 (76.5)	67 (75.3)	
Women	28 (23.5)	22 (24.7)	
Age [n (%)]			0.696†
< 65 years	78 (65.5)	56 (62.9)	
≥ 65 years	41 (34.5)	33 (37.1)	
BMI (kg/m^2)	21.94±2.67	21.73±3.16	0.615‡
Hypertension [n (%)]			0.282†
Yes	15 (12.6)	16 (18.0)	
No	104 (87.4)	73 (82.0)	
Admission VPG (mmol/L)	5.12 (4.75, 5.65)	5.07 (4.64, 5.73)	0.592§
Neoadjuvant chemotherapy [n (%)]			0.750†
Yes	44 (37.0)	31 (34.8)	
No	75 (63.0)	58 (65.2)	
pTNM [n (%)]			0.643†
I/II	63 (52.9)	50 (56.2)	
III/IV	56 (47.1)	39 (43.8)	
Operation approach [n (%)]			<0.0001†
Open gastrectomy	52 (43.7)	85 (95.5)	
Laparoscopic gastrectomy	67 (56.3)	4 (4.5)	
Surgery type [n (%)]			0.022†
Proximal gastrectomy	0	4 (4.5)	
Distal gastrectomy	59 (49.6)	51 (57.3)	
Total gastrectomy	60 (50.4)	34 (38.2)	
POD1 VPG (mmol/L)	5.91 (5.31, 6.78)	6.14 (5.45, 6.99)	0.228§
Pre-SPN HG [n (%)]			0.158†
Yes	5 (4.2)	8 (9.0)	
No	114 (95.8)	81 (91.0)	

VPG: venous plasma glucose; POD1: postoperative day 1; Pre-SPN HG: The returning to the ward capillary blood glucose to ≥11.1 mmol/L more than twice on the operation day; pTNM: pathological tumor–node–metastasis.
†Chi-square test.
‡Independent t-test.
§Mann-Whitney U test.

Table 2. BG concentrations and fluctuations during SPN

	Noninsulin (n=119)	Insulin (n=89)	*p*-value
Mean BGs during SPN (mmol/L)	8.35 (7.45, 9.14)	7.35 (6.67, 8.40)	<0.0001‡
Highest BGs during SPN (mmol/L)	12.3 (10.7, 14.5)	10.4 (8.8, 12.2)	<0.0001‡
BGCR (%)	75.0 (58.3, 90.9)	91.67 (83.3, 100)	<0.0001‡
During SPN Hypoglycemia [n (%)]			0.182§
Yes	0 (0)	2 (2.2)	
No	119 (100)	87 (97.8)	
During SPN HG [n (%)]			<0.0001¶
Yes	58 (48.7)	17 (19.1)	
No	61 (51.3)	72 (80.9)	
Classification of postoperative HG† [n (%)]			<0.0001§
Persistent	3 (2.5)	3 (3.4)	
Improved	2 (1.7)	5 (5.6)	
Late-onset	55 (46.2)	14 (15.7)	
Non-HG	59 (49.6)	67 (75.3)	
CV (%)	28.6 (23.1, 34.1)	21.14 (16.8, 26.3)	<0.0001‡
FCG-CV (%)	18.8 (12.2, 25.9)	14.43 (8.15, 20.3)	0.003‡
LAGE (mmol/L)	7.2 (5.7, 9.7)	5.0 (4.0, 6.7)	<0.0001‡

BG: blood glucose; SPN: supplementary parenteral nutrition; BGCR: blood glucose control rate; During-SPN HG: BG of ≥11.1 mmol/L more than twice during SPN; CV: coefficient of variation; FCG-CV: fasting capillary glucose-coefficient of variation; LAGE: largest amplitude of glycemic excursions.
†The classification of postoperative HG was determined according to HG occurrence before and during SPN.
‡Mann–Whitney U test.
§Fisher's exact test.
¶Chi-square test.

leading to lipid peroxidation and an increase in cardiovascular inflammatory markers, which ultimately increase infection risk and in-hospital complications.[18-20] Steve Kwon et al found that perioperative HG, regardless of whether the patient was diagnosed with diabetes, was associated with nearly twofold risks of higher infection, in-hospital mortality, and operative complications.[2] The same study found that patients with HG but without dia-

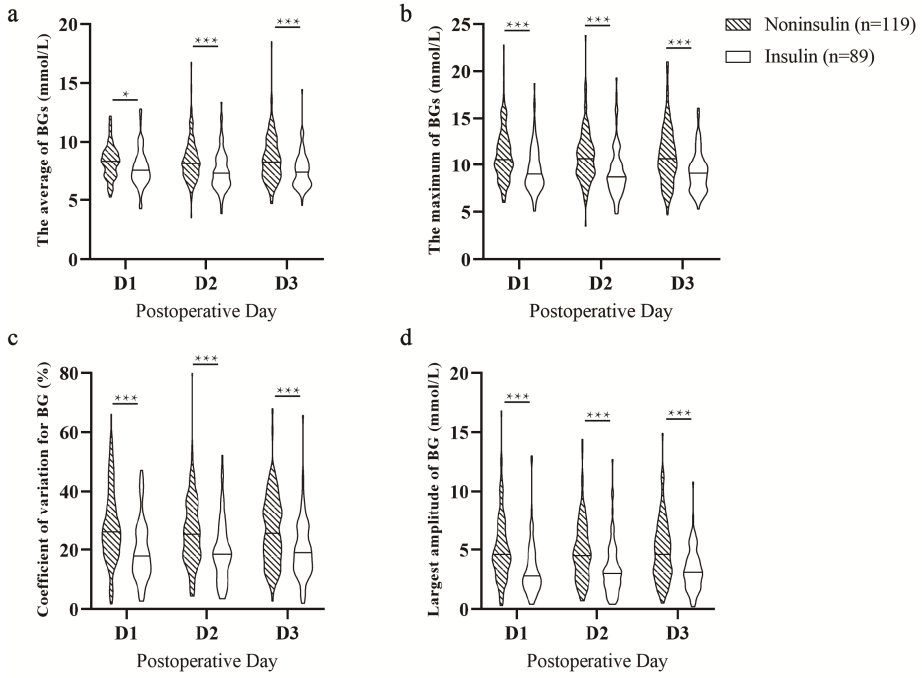

Figure 2. The (a) mean blood glucose (BG), (b) highest BG, (c) coefficient of variation, and (d) fluctuation range on days 1–3 of the supplementary parenteral nutrition period. The violin diagrams show all values, where the black line represents the median. $^{*}p<0.05$, $^{***}p<0.001$.

Table 3. Postoperative complications

	Noninsulin (n=119)	Insulin (n=89)	*p*-value
CI	0 (0, 8.66)	0 (0, 12.3)	0.761†
Postoperative infection			0.608‡
No	97 (81.5)	68 (76.4)	
Grade I–II	19 (16.0)	19 (21.3)	
Grade III–V	3 (2.5)§	2 (2.2)¶	

CI: comprehensive complications index.
†Mann–Whitney U test.
‡Fisher's exact test.
§The three patients were sepsis shock induced by pulmonary infection, severe incision suppuration, and adhesive intestinal obstruction, respectively.
¶The severe infection complications of these two patients were due to abdominal bleeding and anastomotic leakage

Table 4. Hyperglycemia (HG) and postoperative complications

	HG (n=82)	Non-HG (n=126)	*p*-value
CI	8.66 (0, 20.92)	0 (0, 8.66)	0.002†
Postoperative infection			0.324‡
No	61 (74.4)	104 (82.5)	
Grade I–II	19 (23.2)	19 (15.1)	
Grade III–V	2 (2.4)	3 (2.4)	

CI: comprehensive complications index.
†Mann–Whitney U test. ‡Fisher's exact test

Table 5. Relationships between different classifications of postoperative hyperglycemia and complication rates

	Persistent HG (n=6)	Improved HG (n=7)	Late-onset HG (n=69)	Non-HG (n=126)	*p*-value
CI	8.66 (0, 14.20)	0 (0, 22.64)	8.66 (0, 20.92)	0 (0, 8.66)	0.024†
Postoperative infection					0.585‡
No	4 (66.7)	5 (71.4)	52 (75.4)	104 (82.5)	
Grade I–II	2 (33.3)	2 (28.6)	15 (21.7)	19 (15.1)	
Grade III–V	0	0	2 (2.9)§	3 (2.4)¶	

CI: comprehensive complications index; HG: hyperglycemia.
†Mann–Whitney U test.
‡Fisher's exact test.
§The two patients had sepsis shock due to pulmonary infection and severe incision suppuration, respectively.
¶The severe infection complications of these three patients were due to abdominal bleeding, anastomotic leakage, and adhesive intestinal obstruction

betes had worse outcomes compared with patients with diabetes.[2] Claudio Fiorillo et al reviewed the glycemic status in 173 nondiabetic patients after gastrectomy and noted that postoperative HG was a risk factor for increased mortality and complication rates.[3] Ayami Yoneda et al supports that BG improvement prevents surgical site infection (SSI) in nondiabetic patients undergoing gastrointestinal surgery.[21] In our study, insulin therapy prevented HG during SPN, irrespective of HG occurrence before or during SPN, and the CI and infection rate were higher in HG patients than in euglycemic patients, confirming the findings of Claudio Fiorillo et al and Steve Kwon et al. This confirms that glycemic management is crucial in clinical nondiabetic patients.

The optimal target of postoperative BG has always been the focus of discussion. Several studies have indicated that intensive insulin therapy (IIT) targeting BG must be maintained at 4.4–6.1 mmol/L to improve clinical outcomes in different clinical settings.[22] In 2011, Cao et al demonstrated that IIT significantly reduced the postoperative short-term morbidity, but not mortality, among nondiabetic patients receiving PN after D2 gastrectomy, which may be related to insulin sensitivity improvement and increased human leukocyte antigen (HLA)-DR expression on monocytes.[23] Nevertheless, IIT is always associated with undesirable hypoglycemia.[23] As a consequence, most studies have suggested that the random BG of perioperative patients should be controlled within 10.0 mmol/L (180 mg/dL).[5,24] In this study, BGs at 3.9–10.0 mmol/L (70–180 mg/dL) were defined as the standard for effective BG control. BGs of >11.1 mmol/L (200 mg/dL) more than twice was defined as HG, referring to the recommended target value of American society of parenteral and enteral nutrition and the definition of stress-induced HG.[12]

In this study, we observed that nearly half of our patient population had developed HG during hospitalization, regardless of the treatment type. Gianotti et al studied the perioperative BG trend in nondiabetic patients undergoing major elective abdominal surgery and found that the maximum BGs were frequently observed at the end of the surgery.[4] We predict that HG occurrence in our patients was a comprehensive result of surgical stress, decreased physical activity, and SPN. Surgery and trauma increase the levels of counter regulatory hormones, such as glucagon, epinephrine, cortisol, and growth hormone, which collectively result in alterations in carbohydrate metabolism, including insulin resistance (IR), increased hepatic glucose production, impaired peripheral glucose utilization, and relative insulin deficiency.[18] These hormones in the stress setting lead to enhanced lipolysis and increased fatty acid concentration,[25] which also produce dose-dependent IR in peripheral tissues and increase hepatic glucose output in both diabetic and nondiabetic individuals.[12,26] Furthermore, bed rest has been demonstrated to diminish glucose uptake and insulin signaling by insulin-dependent tissues.[27] The patients in this study were on combined nutrition with oral intake and enteral and parenteral feeding. The individualized SPN provides up to 250 g/d of glucose and up to 30% of total calories from lipid emulsions, which may cause glucose overload and hypertriglyceridemia, which contribute to HG. Additionally, the infusion rate is another significant predictor of HG in patients during PN.[28] Although SPN improves patients' nutritional status, the exogenous infusion of glucose and fat emulsion may increase postoperative BG fluctuation.

Significant evidence indicates that both subcutaneous and intravenous insulin are effective in HG management during PN therapy.[28] The addition of insulin to TNA is physiologically convenient and less invasive.[5] However, research addressing the insulin dosage for PN-related HG management in nondiabetic patients is limited. Among these available studies, some suggest the use of 0.5 unit of insulin per 10 g of PN glucose for the management of perioperative HG.[6,29] One study recommended initiating 1 unit of insulin per 10 g of PN glucose when the patient was observed to have serum BG values >10 mmol/L (180 mg/dL) twice consecutively.[30] Insulin dosages were later titrated in increments of 0.05–0.1 unit per 1 g of PN glucose until BG become stable. Two randomized controlled trials have proposed determining the insulin dosage added to PN based on previous day readings and adjusting subcutaneous insulin according to correctional dosing protocol. Addition of insulin subcutaneously and directly had comparable efficacy in controlling BG in both critical and noncritical patients.[7,31] A retrospective study in noncritical adult patients who received general surgeries and postoperative PN recommended the regular addition of insulin to TNA rather than long-acting insulin therapy for a high likelihood of achieving glycemic control.[32] However, robust practical evidence is still lacking to reach a consensus. This study tested the effectiveness of 1 unit of insulin per 6 g of glucose in PN, and this dosage was determined solely according to doctors' experience, which might be a common procedure in Chinese noncritical surgical departments, and the possibility of HG occurrence in perioperative patients has been ignored.

This study has several limitations that need to be taken care of in future research: First, we did not standardize the surgical procedures between the two groups because surgical approaches were determined based on the cancer type. To minimize variances, sensitivity analysis was performed. The relevant outcome indicators of patients undergoing open radical gastrectomy were analyzed, which did not change previous results. Furthermore, a logistic regression analysis of the patients with HG suggested that the choice of surgical procedure was not an independent factor. Second, glycated hemoglobin of patients with known diabetes history was not measured; thus, patients with preexisting diabetes or prediabetes were excluded based on BG at admission, which may not efficiently omit all patients with diabetes. In addition, patients with preexisting IR are more likely to experience HG compared with healthy individuals, irrespective of stress or PN status.[33] However, we were unable to evaluate the IR level of our patient population because of which we cannot rule out the possibility of IR-induced HG during hospitalization. Third, insulin treatment was determined by different attending doctors based on their experiences, as a standard treatment was absent. Therefore, this study was conducted to testify the effectiveness of the currently used therapy.

Conclusion

An increased risk of complications was observed in patients with perioperative HG, which therefore should be prevented carefully and managed effectively. The addition of 1 unit of insulin per 6 g of PN glucose to TNA can improve the overall BG control to a certain extent in patients with cancer receiving postoperative SPN. Further research is needed to make a confirmative recommendation on optimal insulin therapy in patients undergoing surgery for accurate glycemic control.

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AUTHO DISCLOSURES

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Original Article

Diurnal differences in glycemic responses, insulin responses and cognition after rice-based meals

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Background and Objectives: The variation in glycemic responses to white rice caused by the circadian rhythm has been widely investigated but remain controversial. This study investigated diurnal differences in the effect of rice meals on glycemic responses, insulin responses, satiety, and acute cognitive function. **Methods and Study Design:** A total of 20 healthy participants in Group 1 and 14 in Group 2 were served identical servings of cooked white rice containing 50 g of available carbohydrates at 8:00 a.m. (rice at breakfast), 12:30 p.m. (rice at lunch), and 5:00 p.m. (rice at early supper) in a randomized order. Postprandial blood glucose, insulin, satiety, and cognitive performance tests were conducted for each test meal. **Results:** The rice at an early supper elicited significantly milder glycemic responses than did the rice at lunch and resulted in a lower insulin sensitivity than did rice at breakfast. No difference was observed among the test meals in terms of hunger and prospective food intake. Diurnal acute cognitive performance did not differ considerably among the meals. A correlation analysis indicated that low variability in glycemic responses was positively associated with superior cognitive performance. **Conclusions:** A high–glycemic index white rice supper at 5:00 p.m. may facilitate daily glycemic management.

Key Words: glycemic response, diurnal rhythm, white rice, cognitive function, satiety

INTRODUCTION

White rice is among the most common staple foods in East and Southeast Asian countries, and its health effects have been widely investigated yet remain controversial.[1,2] A study demonstrated that individuals who consumed white rice two to three times per week had a lower risk of diabetes than did those who rarely consumed white rice,[3] whereas other studies have observed a positive[4-6] or null[7,8] association between white rice intake and the subsequent incidence of type 2 diabetes. Nevertheless, strategies to control the glycemic response (GR) to polished rice for preventing and managing type 2 diabetes should be developed.

The circadian system regulates metabolism through daily 24-h cycles and plays a major role in regulating glucose, lipid, and energy metabolism.[9,10] Meals consumed in the morning (7:00 a.m.) elicit a milder GR than do those consumed in the afternoon (1:00 p.m.) and evening (7:00 p.m.).[11] A meta-analysis of feeding trials reported that unlike meals consumed early in the day, late-night eating negatively affected the GR, insulin response (IR), and glucose tolerance.[12] In addition, surveys have demonstrated that late-night suppers (8:00 p.m. and later)[13] and higher energy, protein, and fat intake at supper than at breakfast[14] increase the risk of hyperglycemia and type 2 diabetes. However, the diurnal pattern of rice in terms of the GR and IR is yet to be explored.

A moderate increase in the blood glucose concentration is associated with improved learning and memory, partly because of the increased passage of glucose to the brain,[15,16] and a stable glycemic state plays a key role in the prevention of cognitive dysfunction.[17,18] Individuals with long-term impaired blood sugar regulation have an increased risk of Alzheimer's disease and cognitive impairment.[19] However, whether this association is affected by the circadian rhythm is yet to be determined.

In most studies, supper has been scheduled late at night (7:00–8:00 p.m.),[20-23] and the GR to early white rice–based suppers (~5:00 p.m.) has rarely been reported. This study investigated the diurnal difference in the glycemic, insulin, and acute cognitive effects of rice meals. In addition, subjective appetite was evaluated to identify possible side effects. We assumed that (1) a white rice–based supper at 5:00 p.m. would not negatively affect the GR, IR, and subjective appetite and that (2) circadian rhythm would determine the effects of white rice–based meals on acute cognitive performance.

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METHODS

Participant recruitment

Healthy university students aged 20–25 years with a BMI between 18.5 and 24 kg/m^2, a regular sleep–wake cycle, bedtime between 10:00 p.m. and 12:00 a.m., and a regular menstrual cycle (if female) were recruited. Those with a diagnosis of genetic or metabolic diseases (diabetes, impaired glucose tolerance, and hypertension); irregular sleep or eating schedules; dependency on alcohol, tobacco, or drugs; the use of medications or supplements known to affect sleep, the circadian rhythm, or metabolism; eating disorders (bulimia, anorexia nervosa, and binge eating); or participation in competitive or endurance sports were excluded. All eligible individuals who passed duplicated oral glucose tolerance tests provided written informed consent.

Ethics and design

This randomized controlled crossover study was approved by the Ethics Committee of China Agricultural University (ethics number CAUHR-2021011), registered on the Chinese Clinical Trial Registry (ChiCTR2100050541), and conducted in full compliance with the Helsinki Declaration.

Each participant was assigned to three test sessions, with each session being separated by at least 3 days in a randomized order. All test sessions lasted approximately 5 h and were identical in all respects except for food ingestion time. During the sessions, the participants were served meals with cooked white rice at 8:00 a.m. (rice at breakfast, RB), 12:30 p.m. (rice at lunch, RL) and 5:00 p.m. (rice at early supper, RES). The participants were required to arrive at the laboratory 30 min before meal time. They were instructed to schedule and record their daily diet during the test sessions. The trial consisted of two groups. In Group 1, the diurnal postprandial and subsequent-meal glycemic effects were assessed through continuous glucose monitoring along with subjective appetite and acute cognitive function. In Group 2, the diurnal glycemic and insulin effects were investigated through blood collection. The participants were required to follow an identical meal plan the day prior to and after the test sessions.

Test meals

The administered glucose solution contained 55.6 g of dextrose monohydrate powder diluted in 300 mL of water. The meal included cooked rice containing 50 g of available carbohydrates and 184.1 mL of water for weight balance. The rice was prepared in an electric pressure cooker (MY-HT5093, Midea, China). Each serving was prepared using 66.1 g of polished rice (Oryza sativa spp. Japonica, Heilongjiang, China) and 132.2 mL of water. The meals were cooked before each session, immediately served to the participants, and consumed within 15 min to prevent the retrogradation of starch.

Continuous glucose monitoring

Continuous glucose monitors (CGMs; Abbott, Shanghai, China) were inserted under the participants' skin 2 days before the first test and removed 24 h after the last test. The participants were instructed to use the sensor at least once every 8 h in accordance with the manufacturer's instructions. Sensor data were retrospectively stored every 15 min, and occasional missing values (<0.06% of all data) were imputed by averaging adjacent values.[24]

Subjective appetite and acute cognitive function

Subjective appetite, comprising satiety, fullness, hunger, desire to eat, and prospective food intake, was assessed using a visual analogue scale[25,26] before each test meal and at 15, 30, 45, 60, 90, 120, 150, 180, 240, and 270 min after meal ingestion (Figure 1). The Hopkins Verbal Learning Test (HVLT) was used to test short-term listening and memory,[27] the Map Test (MT) was used to test spatial memory,[28] and the visual recall test (VRT) was used to test memory[28] at 30 min before meals and at 60 and 210 min after meal ingestion.

Plasma chemistry

Blood for insulin analysis was obtained through finger pricking by using a sterile, single-use lancing device (Meisheng, China). The participants were encouraged to warm their hands in supplied water before finger pricking and to massage them from the bottom of the palm toward the fingertips to increase blood flow. Before each meal and 15, 30, 45, 60, 90, and 120 min after meal ingestion (Figure 1), 150 μL of capillary blood was collected in Microvette capillary blood collection tubes treated with dipotassium ethylenediamine tetraacetic acid (Lihui Inc., Jiangsu, China) and stored in crushed ice immediately thereafter. The last blood drop was retained for a glucose assay performed using a glucometer (LifeScan Inc., Milpitas, CA, USA). Within 30 min after blood collection, the Microvette tubes with the blood were centrifuged at 1000 × g for 15 min, and 50 μL of the supernatant plasma was pipetted into 0.5-mL Eppendorf tubes (Biosharp Inc., Anhui, China) and stored in a freezer at −80℃ until analysis. The insulin concentration was determined using an ELISA-based test kit (Dogesce Inc., Beijing, China) in accordance with the manufacturer's instructions.

Data processing and statistical analysis

The sample size was verified through calculations in PASS 13 Power Analysis and Sample Size software (NCSS, Kaysville, UT, USA) on the basis of a study in which a 36% reduction in the glycemic index (GI) was

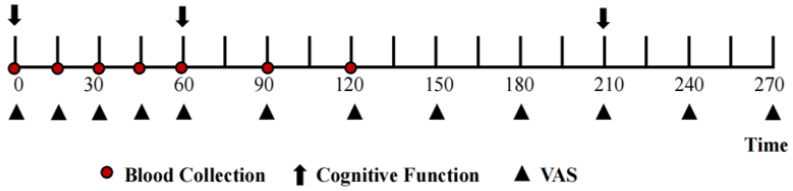

Figure 1. Study design diagram.

observed for whole grain oats and cooked rice compared with the glucose control.[29] If the standard deviation (SD) was assumed to be <15.80, the test would have 85% power to examine a difference (p<0.05) among 13 participants for a GI of 36.

Glycemic and insulin variability were evaluated in terms of the maximum amplitudes of glucose excursion (MAGE),[30] the incremental peak (ΔPeak) and low (ΔLow) of glucose and insulin concentrations, and the positive increments under the curve of GRs and IRs (iAUC).[31] To estimate insulin sensitivity (IS), the homeostatic model of β-cell function (HOMA-B) was calculated as follows: (20 × fasting insulin)/ (fasting glucose − 3.5).[32] IS indices (ISIs) were calculated as follows: 10,000/square root of (fasting glucose × fasting insulin × mean glucose × mean insulin).[33] The insulin secretion sensitivity index-2 (ISSI-2) was calculated as follows: (AUCins/AUCgluc) × ISI.[34]

The results are presented as means (standard errors [SEs]) unless otherwise noted. The Kolmogorov–Smirnov test was performed to check for normal distributions prior to analysis, and a natural logarithmic transformation was used when data were nonnormally distributed. Differences between treatments were identified through ANOVA with Duncan's multiple range test, and statistical significance was set at p<0.05. Correlations among the data were identified through a Pearson's correlation analysis. Statistical analysis was performed using SPSS (version 21.0, SPSS Inc. Chicago, IL, USA).

RESULTS

Participant characteristics

A total of 20 participants in Group 1 and 14 in Group 2 passed the screening and completed all the tests. No adverse events were reported during the test sessions, and all data were included in the analysis. Table 1 lists the participants' baseline characteristics.

GRs from CGMs

Figure 2 presents the GRs to the rice meals indicated by the CGMs. RES led to significantly lower glucose levels at 15, 30, 45, 60, 165, 180, 195, 225, 240, and 270 min than did RB. The GRs to RES were considerably lower than those to RL during the sessions. No significant difference between RB and RL was observed.

Figure 3 presents the GRs to the subsequent and routine meals in Group 1. The lunch after the test breakfast meal elicited significantly higher glycemic increments than did the routine lunch at 15 and 30 min. The blood glucose increments of the supper after the test lunch meal were higher than those of the routine supper at 45, 60, and 75 min. The breakfast after the test supper meal led to an advanced peak and higher glucose increments at 30 and 45 min and lower glucose increments from 90 to 150 min than did the routine breakfast.

GRs and IRs in blood tests

Figure 4 presents the GRs and IRs to the test meals in Group 2. RES elicited significantly lower glycemic in-

Table 1. Participant baseline characteristics

Characteristics	Group 1		Group 2	
	Mean	SD	Mean	SD
Number of participants (male/female)	20 (8/12)		14 (5/9)	
Age (year)	21.8	1.7	22.5	1.9
Body height (cm)	168.3	8.7	166.5	8.0
Body weight (kg)	61.5	10.4	57.4	7.5
BMI (kg/m^2)	21.6	2.2	20.7	2.1
Fat mass (%)	26.0	6.7	25.2	5.5
Basal metabolism rate (BMR) (kcal/day)	1354.2	198.1	1298.5	152.2

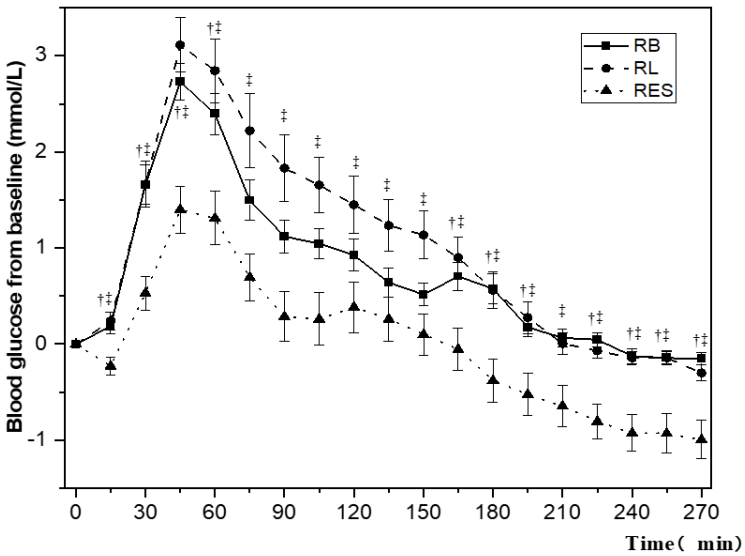

Figure 2. GRs to rice meals (n=20). RB: rice ingested at breakfast (8:00 a.m.); RL: rice ingested at lunch (12:30 p.m.); RES: rice ingested at early supper (5:00 p.m.). †Differences between RB and RES, ‡differences between RL and RES (p<0.05). Values are presented as means, with SE represented by vertical bars.

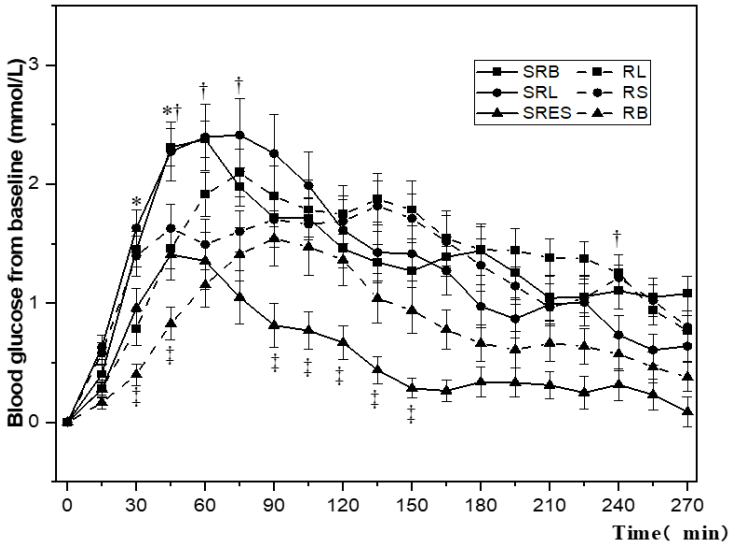

Figure 3. GRs to subsequent and routine meals (n=20). SRB: subsequent meal of rice ingested at breakfast; SRL: subsequent meal of rice ingested at lunch; SRES: subsequent meal of rice ingested at supper; RL: routine lunch; RS: routine supper; RB: routine breakfast. Values are presented as means, with SEs represented by vertical bars. *Differences between SRB and RL, †differences between SRL and RD, ‡differences between SRES and RB (p<0.05).

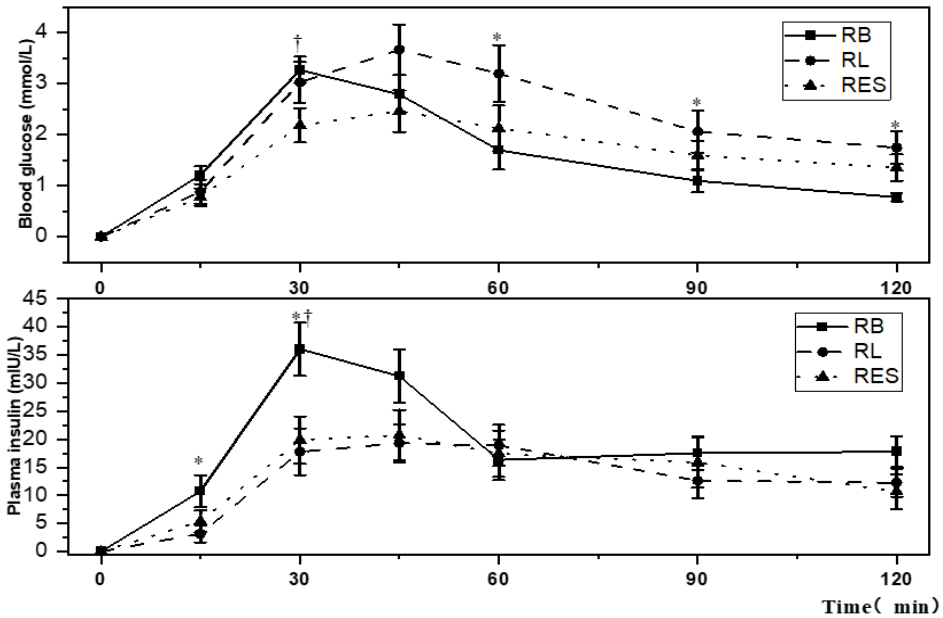

Figure 4. Changes in blood glucose and plasma insulin from baseline for test foods (n=14). RB: rice ingested at breakfast (8:00 a.m.); RL: rice ingested at lunch (12:30 p.m.); RES: rice ingested at supper (5:00 p.m.). Values are presented as means, with SEs represented by vertical bars. *Differences between RB and RL, †differences between RB and RES (p<0.05).

crements than did RB at 30 min, and RB led to lower glucose increments at 60, 90, and 120 min than did RL. The insulin increments of RB were higher than those of RL and RES at 30 min and higher than those of RL at 15 min. No difference in insulin increments between RL and RES was observed.

Table 2 displays the glycemic and insulin variability indices for the test meals. The glycemic peak value, MAGE, and SD of RES were significantly lower than those of RL. RB resulted in higher insulin peak values than did RL and RES. RES increased insulin sensitivity more than did RB in terms of ISI.

Subjective appetite

The RES satiety increments at 90 and 120 min were significantly lower than those of RB and those from 90 to 270 min in relation to those of RL (Figure 5). The fullness of RL was higher than that of RES at 180, 240, and 270 min. The desire to eat for RB was lower than that for RL at 45, 60, and 90 min and lower than that for RES at 120 min. No difference among the test meals in terms of hunger and prospective food intake was observed.

Acute cognitive function

Table 3 presents the VRT results before and after lunch. Significantly more correct numbers in the VRT were observed at −30 min than at 60 min. No significant difference in the VRT results was observed between the breakfast and supper tests or in the results of the MT.

Table 4 presents the results of HVLT before and after

Table 2. Glycemic and insulin variability indices for test meals (mean values and SEs, n=14)

	RB		RL		RES	
	Mean	SE	Mean	SE	Mean	SE
Blood glucose						
ΔPeak (mmol/L)	3.7†‡	0.3	4.2†	0.4	2.8‡	0.4
ΔLow (mmol/L)	-0.1†	0.0	-0.1†	0.1	-0.0†	0.0
MAGE (mmol/L)	3.8†‡	0.3	4.4†	0.3	2.9‡	0.4
SD	1.4†‡	0.1	1.6†	0.1	1.1‡	0.1
$iAUC_{0\text{-}120}$ (mmol/L·min)	192.6†	18.9	277.8†	36.5	197.6†	30.2
Plasma insulin						
ΔPeak (mIU/L)	44.0†	4.1	27.5‡	3.8	29.0‡	4.7
$iAUC_{0\text{-}120}$ (mIU/L·min)	2330.3†	222.6	1598.3†	243.8	1724.2†	345.5
Insulin sensitivity						
HOMA-B	86.8†	6.3	81.1†	6.3	80.8†	5.9
ISI	20.5‡	1.5	23.5†‡	3.0	31.6†	5.3
ISSI-2	267.5†	35.0	203.1†	72.3	315.8†	91.5

RB: rice ingested at breakfast (8:00 a.m.); RL: rice ingested at lunch (12:30 p.m.); RES: rice ingested at supper (5:00 p.m.).
Values are the mean glycemic characteristics of the test meals with their SE.
†‡Significant differences among test meals ($p<0.05$).

Table 3. VRT results before and after lunch (mean values and SE, n=20)

Time (min)	Correct number in VRT (n)
-30	13.21±0.91†
60	9.16±0.81‡
210	12.32±1.06†‡

VRT: Visual Recall Test.
Values are the mean test results with SEs.
†‡Significant differences among time points ($p<0.05$).

each meal. The FT error at −30 min was higher than that at 60 and 210 min for lunch, and the TT error at −30 min was higher than that at 210 min. No differences among the tests at individual time points were observed, but omissions showed a decreasing trend.

Correlation analysis

The correlation analysis indicated that the glycemic MAGE had a significant and positive correlation with the number of errors in three HVLTs and with the number of category C errors at −30 min in the MT (Table 5). The glycemic peak value and $iAUC_{0\text{-}270}$ were positively correlated with the number of category B errors at 60 min in the MT. A negative correlation between $iAUC_{0\text{-}270}$ and the correct number at 60 min in the VRT was observed.

DISCUSSION

The early (5:00 p.m.) supper elicited a milder GR than did the lunch (12:30 p.m.) and reduced IS more than did the breakfast (8:00 a.m.) with the same amount of white rice containing 50 g of available carbohydrates. The satiety responses and diurnal acute cognitive performance did not differ considerably among meals. However, the results suggested that stable GRs were positively associated with superior cognitive performance.

The CGM and capillary blood tests consistently indicated that RES produced lower ΔPeak (−1.9 mmol/L for CGM and −1.4 mmol/L for plasma blood) and MAGE (−1.2 mmol/L for CGM and −1.5 mmol/L for plasma blood) values than did RL. In addition, RES led to a lower insulin ΔPeak and higher IS than did RL.

The results suggested that if served early, a high-carbohydrate supper may not necessarily lead to a considerable surge in blood glucose or low insulin sensitivity, as observed in other studies.[12] The inconsistency between the results of this study and those of others can be explained by several factors.

First, the prescribed supper times in other studies have been 7:00 p.m.,11 7:30 p.m.,[21] 8:00 p.m.,[22] and 10:00 p.m.,[23] whereas it was 5:00 p.m. in this study. One study demonstrated that the GRs to supper at 6:00 p.m. were significantly lower than those to supper at 10:00 p.m.; thus, earlier supper time may lead to more stable GRs.[23]

Second, unlike meals in others studies, which consisted of multiple foods, the meals in this study consisted of rice and were thus low protein, low fat, and high carbohydrate. One study reported that consuming mostly protein during the day and mostly carbohydrates at night for 8 weeks led to a nonsignificant decrease in fasting blood glucose in individuals with overweight and obesity.[35] An epidemiological study reported that consuming more total energy, total fat, and protein but not carbohydrate at supper than at breakfast increased the risk of diabetes, cardiovascular disease, and all-cause mortality.[36] Studies have attributed the hypoglycemic effect of high-GI suppers to an increase in insulin levels.[37] However, in this study, the postprandial insulin level of RES was lower, and the IS was higher than that of RB.

Third, this study limited the window of mealtime to a 9-h period. An early time-restricted feeding pattern might have contributed to stable GRs at night.[38] Because most adults work from 9:00 a.m. to 5:00 p.m., the only practical window for mealtime would be breakfast at 8:00 a.m. to supper at 5:00 a.m. In addition, dinner service at uni-

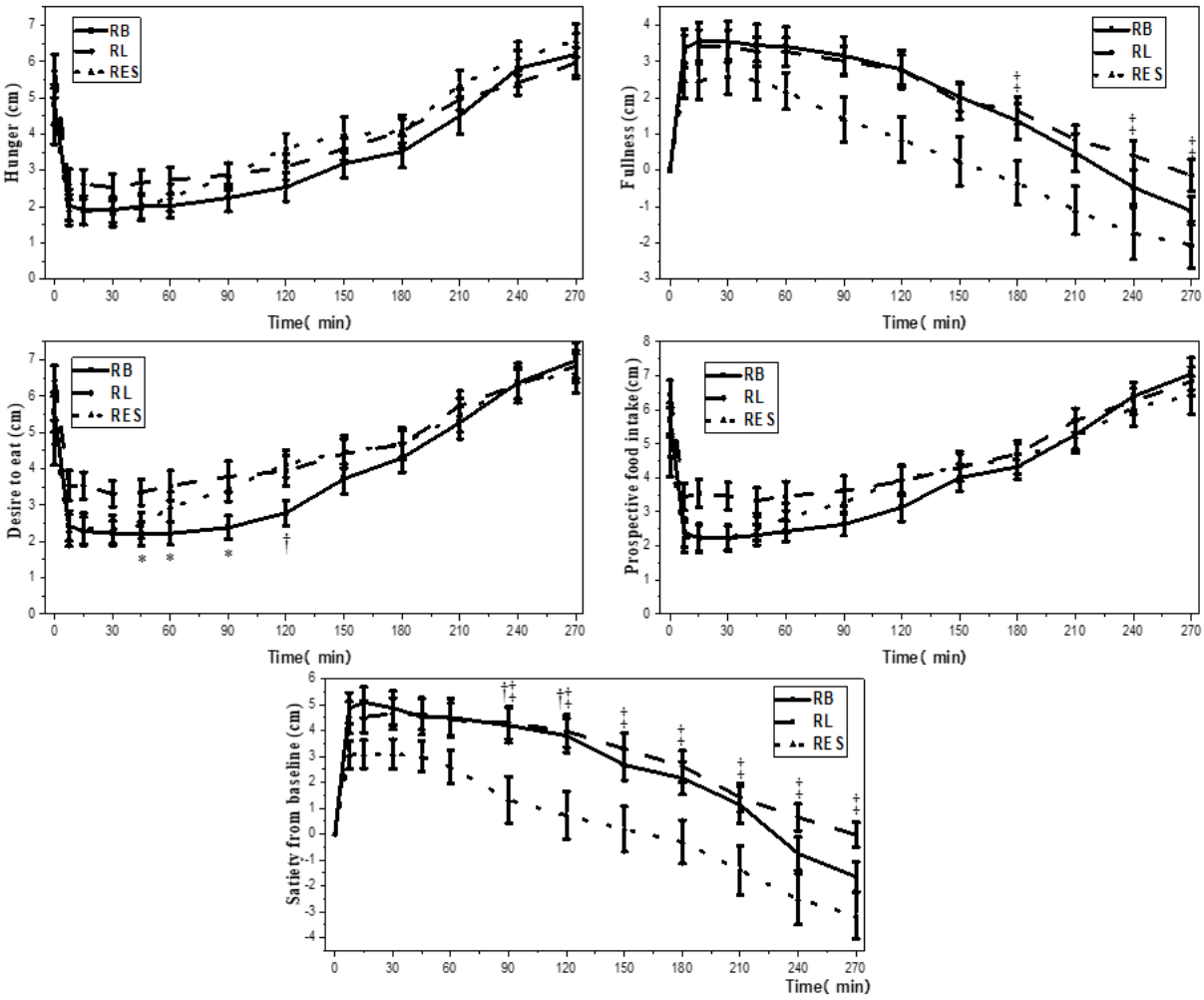

Figure 5. Changes in satiety from baseline for test foods assessed using a visual analogue scale (n=20). RB: rice ingested at breakfast (8:00 a.m.); RL: rice ingested at lunch (12:30 p.m.); RES: rice ingested at supper (5:00 p.m.). *Differences between RB and RL, †differences between RB and RES, ‡differences between RL and RES (p<0.05).

versity cafeterias and restaurants usually begins at 5:00 p.m.

When comparing GRs to the subsequent and routine meals, we observed that GRs to the supper after the test lunch were more severe than those to the routine suppers, whereas GRs to the breakfast after the test supper did not differ considerably from those to the routine breakfast. This result raised questions regarding the second-meal effect of high-GR meals[39] because the test meal at lunch led to the most severe GRs. Strong GRs after lunch might explain sleepiness after lunch; one study revealed that those without diabetes who nap during the day have high HbA1c levels and strong IRs.[40] The possible effects of lunchtime carbohydrate intake on postprandial GRs and after-meal drowsiness must be investigated.

Studies have demonstrated that early suppers do not significantly increase hunger scores.[41] In this study, the correlation analysis indicated a null association between GRs and satiety indicators. Although the GRs of RES were significantly lower, the three test meals resulted in similar levels of self-reported desire to eat. Therefore, a stable postprandial blood glucose pattern may help prevent hunger at night.[42]

No significant diurnal difference in acute cognitive function was observed, but the correlation analysis revealed that severe postprandial glycemic fluctuations were correlated with decreased short-term listening, spatial memory, and graphical memory. Studies have indicated that the key factor determining the effect of glucose on cognitive ability is not the concentration of glucose but the glycemic pattern after the release of glucose.[43,44] Variability in glycemic levels (rather than the absolute concentration of blood glucose) is crucial to the regulation of cognitive function.[45]

To the best of our knowledge, this is the first study to investigate diurnal differences in the effects of white rice meals on GRs, IRs, and acute cognitive function. Because white rice is a global staple food, the GRs, proper consumption time, and cognitive effects of white rice should be investigated. This study revealed that scheduling suppers at 5:00 p.m. and limiting mealtimes to a 9-h window could provide glycemic solutions for high-GI food.

Because this study was conducted as an acute trial in healthy young volunteers, the applicability of the results to those with impaired glucose tolerance, diabetes, and other health conditions must be determined. The long-term effects and underlying mechanisms should be investigated through longer interventions and analyses of hormones such as glucagon-like peptide-1 and gastric inhibitory polypeptide.

Table 4. Results of HVLT before and after breakfast, lunch and dinner (mean values and SE, n=20).

Time (min)	FT error (n)	FT omission (n)	ST error (n)	ST omission (n)	TT error (n)	TT omission (n)
Breakfast						
-30	0.37±0.14†,††	4.42±0.40†,§	0.21±0.10†,††	1.79±0.44†,¶	0.11±0.07†,††	0.37±0.16†,††
60	0.37±0.16†,††	5.47±0.28†,§	0.23±0.13†,††	2.58±0.48†,¶	0.21±0.10†,††	1.00±0.28†,††
210	0.21±0.10†,‡‡	5.26±0.33†,§	0.21±0.10†,‡‡	1.95±0.36†,¶	0.16±0.09†,‡‡	0.95±0.31†,††
Lunch						
-30	0.47±0.12†,††	5.42±0.54†,§	0.37±0.11†,††	2.32±0.47†,¶	0.32±0.11†,††	1.21±0.38†,††
60	0.05±0.05†,††	5.05±0.46†,§	0.11±0.07†,††	2.21±0.52†,¶	0.16±0.09†,††	1.32±0.39†,¶
210	0.16±0.12‡,‡‡	5.68±0.32†,§	0.32±0.13†,‡‡	2.58±0.38†,¶	0.26±0.15†,‡‡	1.21±0.42†,††
Supper						
-30	0.21±0.10†,‡‡	6.26±0.23†,§	0.26±0.10†,‡‡	2.79±0.35†,¶	0.42±0.16†,‡‡	1.42±0.25†,††
60	0.21±0.10†,††	4.84±0.44‡,§	0.21±0.12†,††	1.68±0.47†,¶	0.21±0.12†,‡,††	1.11±0.43†,¶,††
210	0.16±0.09†,‡‡	6.05±0.35†,§	0.11±0.07†,‡‡	2.74±0.41†,¶	0.05±0.05†,‡‡	1.58±0.41†,††

FT error: the error number in the first test; FT omission: the omission number in the first test; ST error: the error number in the second test; ST omission: the omission number in the second test; TT error: the error number in the third test; ST omission: the omission number in the third test.
Values are the mean test results with SE.
†,‡ Significant differences among time points (p<0.05).
§,¶,††,‡‡ Significant differences among the tests at the same time point (p<0.05).

Table 5. Correlation between postprandial glucose and satiety in supper.

Glycemic characterastics	-30min FT error in HVLT (n)	210min ST error in HVLT (n)	210min TT error in HVLT (n)	-30minC-error in MT (n)	60minB-error in MT (n)	60min correct number in VRT (n)
ΔPeak (mmol/L)	0.935	0.935	0.945	0.915	1.000*	-0.993
MAGE (mmol/L)	1.000*	1.000*	1.000**	0.998*	0.948	-0.974
$iAUC_{0\text{-}270}$ (mmol·min/L)	0.958	0.958	0.966	0.941	0.999*	-0.999*

FT error: the error number in the first test; ST error: the error number in the second test; TT error: the error number in the third test; C-error: area name wrongly recalled; B-error: correct name placed in the wrong area.
$^{*}p$<0.05, ** p<0.01.

Conclusion

Our study revealed that consuming white rice containing 50.0 g of available carbohydrates at 5:00 p.m. for supper resulted in stable GRs, increased insulin sensitivity, and similar satiety level, subsequent-meal GRs, and cognitive performance. High–glycemic index white rice suppers at 5:00 p.m. may facilitate daily glycemic management.

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AUTHOR DISCLOSURES

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Original Article

Lactobacillus casei modulates inflammatory cytokines and metabolites during tuberculosis treatment: A post hoc randomized controlled trial

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Background and Objectives: Inflammatory cytokines and metabolic abnormalities are common in patients with tuberculosis. Observational studies have indicated that probiotics modulate inflammatory cytokines and metabolites; however, clinical evidence of the effect of probiotics on patients with tuberculosis is lacking. This study investigated the effects of *Lactobacillus casei* on inflammatory cytokines and metabolites during tuberculosis treatment. **Methods and Study Design:** A randomized controlled trial was conducted. A total of 47 inpatients were included and randomly assigned to receive standard antituberculosis therapy only (control group) or that treatment together with 1×10^{10} colony-forming units per day of *Lactobacillus casei* (low-dose group) or 2×10^{10} colony-forming units per day of *Lactobacillus casei* (high-dose group) for 4 weeks of intensive treatment during hospitalization. Plasma samples were analyzed for inflammatory cytokines and metabolomics with ELISA kits and ultrahigh performance liquid chromatography quadrupole time-of-flight mass spectrometry. **Results:** Daily *Lactobacillus casei* supplementation of up to 2×10^{10} colony-forming units significantly lowered the concentrations of tumor necrosis factor-α, interleukin-6, interleukin-10, and interleukin-12 (p=0.007, p=0.042, p=0.002, p<0.001, respectively) in patients with tuberculosis. Compared with the control and low-dose groups, the plasma metabolites of phosphatidylserine, maresin 1, phosphatidylcholine, L-saccharopine, and pyridoxamine were significantly upregulated, and N-acetylmethionine, L-tryptophan, phosphatidylethanolamine, and phenylalanine were downregulated in the high-dose group. Strong correlations were observed between metabolites and inflammatory cytokines. **Conclusions:** *Lactobacillus casei* supplementation during the intensive phase of tuberculosis treatment can significantly modulate inflammatory cytokines and metabolites. Decreased inflammatory cytokines may be related to metabolite changes.

Key Words: probiotics, tuberculosis, inflammatory cytokines, metabolites, randomized controlled trial

INTRODUCTION

Pulmonary tuberculosis (TB) is a disease caused by the bacterium *Mycobacterium tuberculosis*. The global TB burden was approximately 10 million, and the mortality was approximately 1.2 million in 2019.[1] China also has a high TB burden; in 2019, 833,000 people were diagnosed with TB, and 31,000 patients died of it.[1]

Currently, a combination of four first-line antimycobacterial drugs (isoniazid, rifampicin, ethambutol, and pyrazinamide) are used to treat TB clinically.[2] All four drugs are prescribed to patients during the intensive phase—the first 2 months of TB treatment.[3] Clinical treatment generally produces favorable therapeutic effects. However, inflammatory cytokines and an individual's metabolic profile can be altered after an infection of *M. tuberculosis*.[4,5] Studies have indicated that compared with healthy individuals, those with TB exhibit significantly higher mRNA expressions of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6).[6] Additionally, studies on systemic metabolites have indicated that the abundance of tryptophan, alanine, phosphatidylcholine (PC), and phosphatidylethanolamine (PE) are altered in patients with TB, compared with healthy individuals.[5]

Nutritional approaches are available to improve health.[7,8] Probiotics are defined as "live microorganisms that when administered in adequate amounts, confer a health benefit on the host."[8] Several studies have indicated that the consumption of probiotics can regulate metabolites.[9,10] Supplementation with probiotics, including *Lactobacillus casei* (*L. casei*; 7×10^9 colony-forming units [CFU] per day), can regulate an individual's plasma metabolic profile and increase total plasma glutathione.[11]

Additionally, probiotics can reduce the concentrations

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of anti-inflammatory cytokines.[12] Studies have indicated that metabolites are related to inflammatory cytokines.[13] However, an extensive literature search indicated that no studies have focused on the effects of probiotics on the plasma inflammatory cytokines and metabolites of patients receiving TB treatment.

L. casei is a safe, well-understood probiotic species with the approval and recognition of the United States Food and Drug Administration. *L. casei* provides health-promoting effects, including improving gastrointestinal dysfunction, preventing colorectal tumors, and suppressing cholestasis-related liver indices.[14-16] This study involved a post hoc randomized controlled trial (RCT) to examine the effects of *L. casei* on the plasma inflammatory cytokines and metabolites of patients with TB during intensive treatment. Correlations between inflammatory cytokines and metabolites were also explored.

METHODS

Study design and participants

This RCT was conducted with hospitalized adult patients at a chest hospital in Shandong, China, from December 2017 to January 2019. A total of 429 patients with TB were enrolled in the trial; 10 patients withdrew, 9 patients presented adverse gastrointestinal symptoms, and 13 patients were lost to follow-up (Figure 1). Because of the low availability of plasma samples, inflammatory cytokines and metabolomics were measured in 47 patients, who were simple-randomly allocated to three groups. A total of 16 patients were included in the low-dose *L. casei* group (1×10^{10} CFU daily), 16 in the high-dose *L. casei* group (2×10^{10} CFU daily), and 15 in the control group (without *L. casei* intervention). All patients received TB treatment during the 4 weeks of supplementation.

The trial was performed in accordance with the Declaration of Helsinki, approved by the Ethics Committee of Qingdao Center of Disease Control and Prevention (201703), and registered at the China Clinical Trial Registry Center (ChiCTR-IOR-17013210). All participants provided written informed consent and permission to use their blood samples for this study.

Diagnostic criteria

The inclusion criteria were age 18–65 years, agreement to participate and provide written consent, and a diagnosis of pulmonary TB, which was based on compatible clinical symptoms (e.g., cough, hemoptysis, weight loss, fever, and night sweat) with a computed tomography scan and sputum smear test, as recommended by the WHO,[17] at clinical examination. The exclusion criteria were a diagnosis of extrapulmonary TB (e.g., enterophthisis and bone TB); self-reported cardiovascular disease, diabetes, hematological disease, gastrointestinal disease, liver malfunction, tumor, severe mental or psychological illness, or cognitive impairment; the use of probiotic supplementation within the previous 2 months; or incomplete information.

Randomization and intervention

All participants were inpatients during the first 4 weeks of supplementation. They received standard TB treatment with a combination of four antibiotics (isoniazid, rifampicin, ethambutol, pyrazinamide) and were randomly allocated to three groups. The allocation sequence was generated by an independent investigator using an online randomization generator (http://www.randomization.com). The study was an open-label randomized controlled study, and allocation was unmasked.

The *L. casei* was prepared in liquid through a commercial probiotic drink from Yakult Corporation (Tokyo,

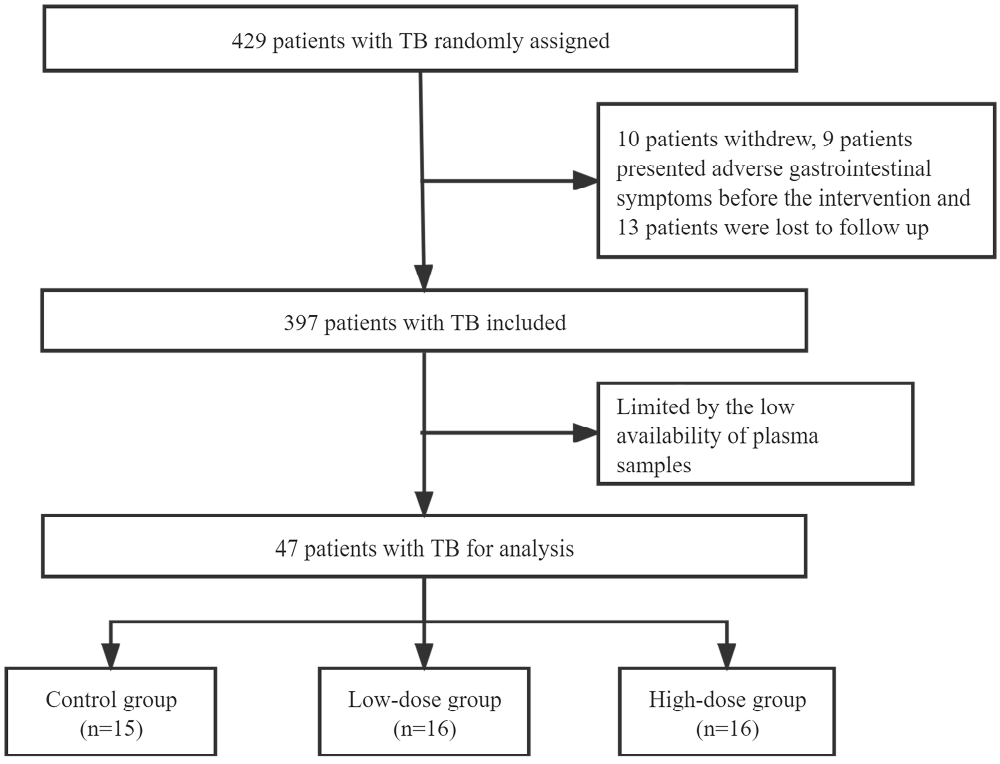

Figure 1. The trial flow chart.

Japan). The probiotic drink contained the *L. casei* strain Shirota, filtered water, skimmed milk powder, glucose, and sucrose. Each bottle (100 mL) provided approximately 10 billion CFU of *L. casei* Shirota, 68.5 kcal of energy, 1.2 g of protein, and 15.7 g of carbohydrates. The probiotic drinks were allocated to patients twice per month. Patients were instructed to shake the bottles before consumption and consume the *L. casei* 30–60 min after meals. The first-month intervention was conducted during the patients' hospitalization period. Compliance was assessed through personal interviews and returned empty bottles.

Data collection and plasma treatment

In the baseline clinical assessment, participants' demographic information was collected, their clinical symptoms were assessed, and they underwent chest radiography; moreover, sputum and blood samples were collected. The blood samples were used to determine the white blood cell differential count (Medical Record) and cytokines concentrations (ABclonal Technology's ELISA kits; Wuhan, China). Symptoms and signs were recorded using a standard questionnaire to calculate TBscores before antituberculosis therapy. TBscore can be used to quantify the severity of TB and the included symptoms and signs were cough, hemoptysis, sputum production, dyspnea, chest pain, night sweat, fatigue, loss of appetite, fever and BMI.[18] Presence of each of the first 9 symptoms and signs scored 1 point. A BMI of less than 16 kg/m^2 scored 2 points, 16-18 kg/m^2 scored 1 point, more than 18 kg/m^2 scored 0 point. The range of TBscore was 0–11 points.

For the ultrahigh performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC Q-TOF LC/MS) analysis, 50 μL of the plasma sample from each patient was transferred to an EP tube and mixed with 250 μL of prechilled acetonitrile. The mixture was then vortexed for 1 min, incubated on ice for 15 min, and centrifuged at 15,000 rpm for 15 min at 4°C. A total of 100 μL of supernatant was removed and filtered with a 0.22-μm organic filter membrane for UHPLC Q-TOF LC/MS analysis.

UHPLC Q-TOF LC/MS

UHPLC Q-TOF LC/MS analysis was performed on the metabolites in the plasma samples using Agilent 1290 Infinity II—UHPLC (Agilent, USA) coupled with Agilent 6530 Q-TOF LC/MS (Agilent, USA). The ACQUITY UPLC BEH C18 column (100 × 2.1 mm^2, 1.7 μm) was the model of chromatographic separation, and the column temperature was set at 20°C, with an injection volume of 2 μL. Separation was performed at a flow rate of 0.4 mL/min under a gradient program in which mobile phase A was composed of water containing 0.1% formic acid (v/v), and mobile phase B was composed of acetonitrile. The elution gradient was set as follows: 0 min, 95% B; 3 min, 80% B; 6.5 min, 50% B; 12.5 min, 15% B; and 17.5 min, 0% B. The stop time was 23 min. The mass spectrometry conditions were as follows: the electrospray ion source was detected using positive ion mode, sheath and auxiliary gases were both nitrogen, mass scanning range was 50–1,000 m/z with a scan time of 0.2 s and scan rate of 1 spectra/s, and capillary and sampling cone voltages were 3 kV and 40 V, respectively.

Statistical analyses

The chi-square test and Kruskal–Wallis H test were adopted for an analysis of baseline characteristics of the study population. Inflammatory cytokine data were log-transformed and analyzed with ANOVA. Peak intensities of metabolites were analyzed with a nonparametric test (p<0.05) with a Dunn's multiple comparisons test conducted between groups. The Spearman nonparametric test was used to analyze the correlations between inflammatory cytokines and metabolites. Mass spectrometry data were further processed through normalization, scaling, filtering, and statistical analysis using MetaboAnalyst 5.0 (http://www.metaboanalyst.ca). The orthogonal partial least squares discrimination analysis (OPLS-DA) model was used to perform analysis between groups with a permutation test to assess the risk of overfitting the model. A fold change (FC) of >1.2 or <1/1.2 and a false discovery rate (FDR) of <0.05 were used to evaluate differential metabolites. Inflammatory cytokine concentrations, shared metabolites peak intensity, and a correlation heat map were illustrated using Graphpad Prism 8.0.2 software. Venn diagrams were constructed by http://bioinformatics.psb.ugent.be/webtools/Venn/. The chi-square test, Kruskal–Wallis H test, and ANOVA were conducted with SPSS 26.0.

RESULTS

Clinical characteristics of the study population

In this study, 47 patients with TB were recruited between December 2017 and January 2019. A total of 15 patients were randomly assigned to the control group, 16 to the low-dose probiotics group, and 16 to the high-dose probiotics group. Patients' clinical characteristics are presented in Table 1. Baseline information comprising age, sex and BMI were comparable among the three groups (p>0.05). The signs and symptoms of the patients were recorded and quantified as TBscore.[18] TBscore levels did not significantly differ among the three groups (p>0.05).

Effects of probiotics on white blood cell differential count and inflammatory cytokines

The white blood cell differential count and inflammatory cytokine concentrations after a 4-week follow-up are presented in Table 2. The numbers of neutrophils, lymphocytes, monocytes, and eosinophils or concentrations of interferon-γ (IFN-γ; p=0.912) did not differ significantly among the control, low-dose, and high-dose groups. However, the concentrations of TNF-α, IL-6, interleukin-10 (IL-10), and interleukin-12 (IL-12) in the high-dose group were significantly lower than in the control and low-dose groups (p<0.05; Figure 2). The TNF-α, IL-6, IL-10, and IL-12 concentrations were similar in the control and low-dose groups (Figure 2).

Metabolomic alteration between the control group and probiotic group

The plasma metabolites changed significantly between the control and high-dose groups, but no significant differences were observed between the control and low-dose

Table 1. Baseline characteristics of patients with tuberculosis†

	Control group (n=15)	Low-dose group (n=16)	High-dose group (n=16)	*p*
Age	33.5 (15.2)	26.1 (10.8)	26.1 (10.6)	0.171
Sex (male)	8 (53.3%)	11 (68.8%)	8 (50.0%)	0.521
Body-mass index, kg/m^2	21.2 (3.4)	20.6 (2.8)	20.0 (2.4)	0.581
TBscore	3.00 (1.25, 4.00)	2.00 (1.25, 3.75)	4.00 (1.25, 4.00)	0.525

†Numerical variables are presented as mean ± standard deviation for normally distributed variables or median (interquartile range) for non-normally distributed variables. Categorical variables are presented as number (percentage).

Table 2. The plasma white blood cell differential count and inflammatory cytokine concentrations after a 4-week follow-up†

	Control group (n=15)	Low-dose group (n=16)	High-dose group (n=16)	*p*
White blood cell count (10^9/L)	5.20 (3.86-6.74)	5.70 (5.04-6.85)	6.16 (4.39, 7.51)	0.580
Neutrophil count (10^9/L)	2.95 (2.21-4.22)	3.34 (2.50-4.36)	3.34 (2.47, 4.63)	0.733
Lymphocyte count (10^9/L)	1.42 (0.96-1.87)	1.60 (1.12-1.95)	1.91 (1.42, 2.35)	0.210
Monocyte count (10^9/L)	0.53 (0.46-0.65)	0.62 (0.40-0.77)	0.55 (0.43, 0.67)	0.798
Eosinophil count (109/L)	0.19 (0.11-0.24)	0.19 (0.12-0.36)	0.10 (0.06, 0.23)	0.113
IFN-γ lg (pg/mL)	0.53 (0.22)	0.52 (0.17)	0.50 (0.25)	0.912
TNF-α lg (pg/mL)	0.91 (0.36)	0.88 (0.18)	0.60 (0.36)	0.012
IL-6 lg (pg/mL)	0.51 (0.58)	0.53 (0.46)	0.11 (0.48)	0.057
IL-10 lg (pg/mL)	0.91 (0.36)	0.98 (0.21)	0.42 (0.54)	0.001
IL-12 lg (pg/mL)	1.04 (0.22)	1.07 (0.10)	0.72 (0.29)	<0.001

†Inflammatory cytokine data were log-transformed and analyzed with ANOVA. Plasma white blood cell differential count are presented as median (interquartile range) and inflammatory cytokine concentrations are presented as mean±standard deviation

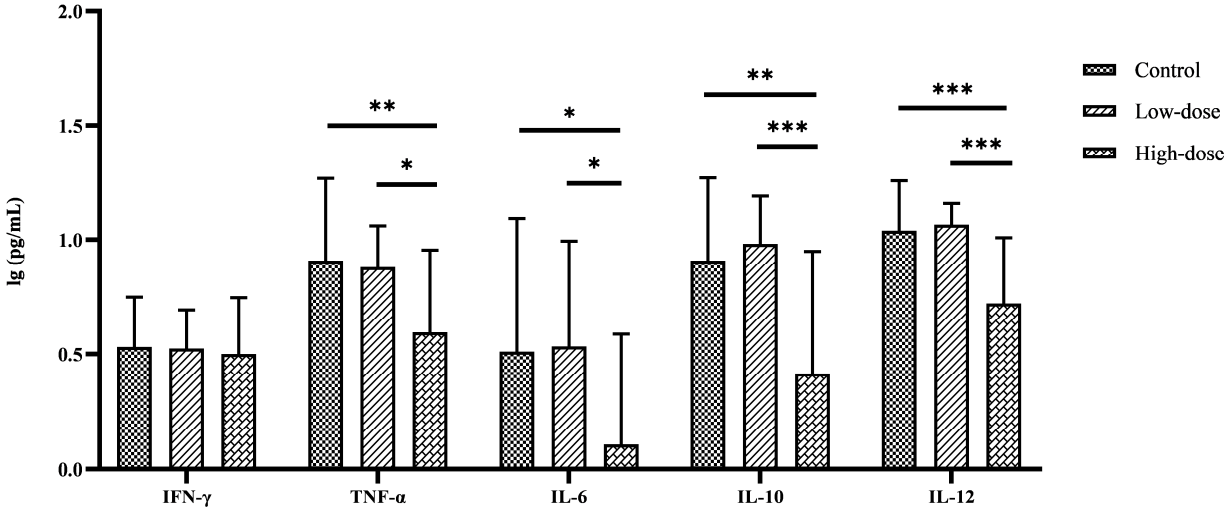

Figure 2. *L. casei* intervention reduced inflammatory cytokine concentrations in patients with tuberculosis. Inflammatory cytokine data were log-transformed and analyzed with ANOVA. $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

groups. The metabolites in the control and high-dose groups were clearly separated by the OPLS-DA model (Figure 3a). One thousand permutation tests yielded an R^2Y value of 0.977 (p=0.049) and a Q^2 value of 0.553 (p<0.001) between the control and high-dose groups (Figure 3b), suggesting model reliability with no evidence of overfitting. Using an FC cutoff value of >1.2 or <1/1.2 and an FDR cutoff value of <0.05, 44 differential metabolites were identified in patients in the high-dose group (in relation to the control group), of which 22 were upregulated and 22 were downregulated (Figure 3c, Supplementary table 1). The primary upregulated metabolites in the high-dose group (in relation to the control group) were pyridoxamine, histidine, N-3-oxo-dodecanoyl-L-Homoserine lactone (3-oxo-C12-HSL), phosphatidylserine (PS), maresin 1 (MaR1), and PC. The primary downregulated metabolites were N-acetylmethionine; 11, 12 epoxyeicosatrienoic acid (11, 12-EET); L-tryptophan; and PE.

Metabolomic alteration between the low-dose group and the high-dose group

Results of OPLS-DA demonstrated that the plasma samples of patients with TB in the low-dose and high-dose groups were clearly separated, suggesting the probiotics caused significant changes in their metabolic profiles (Figure 4a). One thousand permutation tests yielded an R^2Y value of 0.983 (p=0.007) and a Q^2 value of 0.665

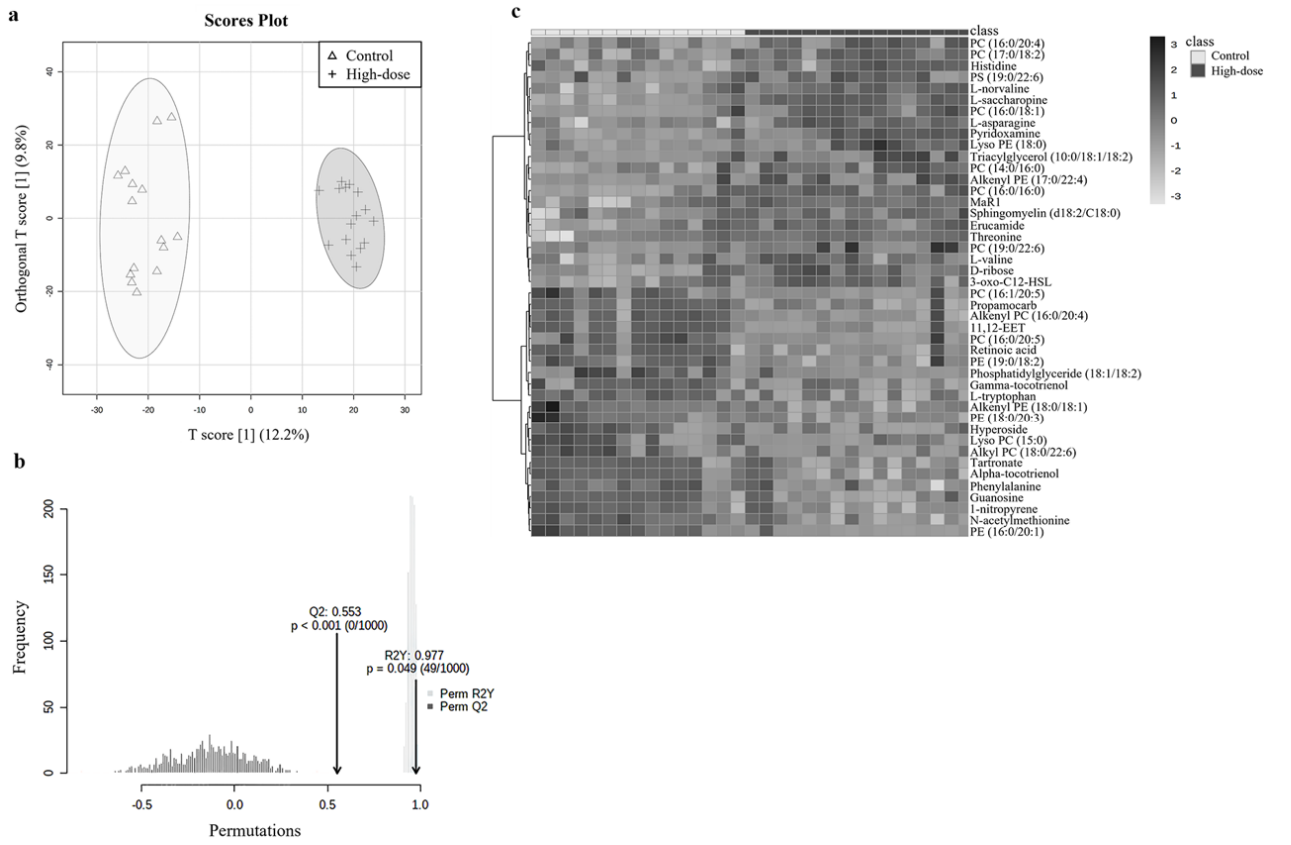

Figure 3. Metabolomic alteration between the control and high-dose groups. (a) The metabolites in the control and high-dose groups were clearly separated by the orthogonal partial least squares discrimination analysis model. (b) The model had no evidence of overfitting. One thousand permutation tests yielded an R^2Y value of 0.977 (p=0.049) and a Q^2 value of 0.553 (p<0.001). (c) Heat map of differential metabolites in the high-dose group compared with the control group. The shades of the color represented metabolites levels (black, and white indicated higher level, and lower level, respectively).

(p<0.001; Figure 4b) indicating no overfitting. With cut-offs of FC >1.2 or <1/1.2 and FDR <0.05, 49 differential metabolites were identified in the high-dose group (in relation to the low-dose group), of which 29 were upregulated and 20 were downregulated (Figure 4c, Supplementary table 2). In patients in the high-dose group, the upregulated metabolites comprised L-valine, linoleic acid, L-asparagine, MaR1, 3-oxo-C12-HSL, pyridoxamine, PC, and PS. The downregulated metabolites comprised PE, L-tryptophan, and N-acetylmethionine.

Changes of key differential metabolites after L.casei suppplementation

According to the FC and FDR, 32 metabolites were commonly identified as differential metabolites through comparison among the control, low-dose, and high-dose groups (Figure 5a). Among the three groups, 11 metabolites exhibited dramatic changes. Compared with the control and low-dose groups, the high-dose group exhibited significant upregulation of pyridoxamine, L-saccharopine, PS (19:0/22:6), MaR1, PC (16:0/20:4), PC (16:0/18:1), and PC (16:0/16:0) (Figure 5b–5h). Phenylalanine, N-acetylmethionine, PE (16:0/20:1), and L-tryptophan, however, were downregulated in the high-dose group (Figure 5i–5l).

Differential metabolites correlate with inflammatory cytokines

To examine potential associations between metabolites and inflammatory cytokines, Spearman correlation analysis was performed. A heat map of the scaled correlations was generated between the metabolites and identified inflammatory cytokines (Figure 6). Strong correlations were observed between PS (19:0/22:6) and TNF-α (r=−0.507, p<0.001), IL-10 (r=−0.573, p<0.001), and IL-12 (r=−0.528, p<0.001); between L-tryptophan and IL-12 (r=0.553, p<0.001); between PC (16:0/18:1) and TNF-α (r=−0.403, p=0.005); between PC (16:0/16:0) and IL-12 (r=−0.467, p=0.002); and between PE (16:0/20:1) and TNF-α (r=0.439, p=0.002).

DISCUSSION

The present study is the first RCT to investigate the effects of probiotics on plasma inflammatory cytokines and metabolites in patients with TB. The results indicated that daily *L. casei* supplementation during TB treatment modulated inflammatory cytokines and metabolites in plasma. Spearman correlation analysis revealed strong correlations between several inflammatory cytokines and metabolites.

The authors' previous studies have revealed the beneficial effect of *L. casei* on the composition of gut microbiota[16] and that the circulating metabolites affect gut microbiota composition.[19] The present RCT was conducted on the basis of the aforementioned results and revealed that *L. casei* supplementation regulated the abundance of MaR1, L-tryptophan, N-acetylmethionine, PS, PC and the concentrations of TNF-α, IL-6, IL-10, IL-12.

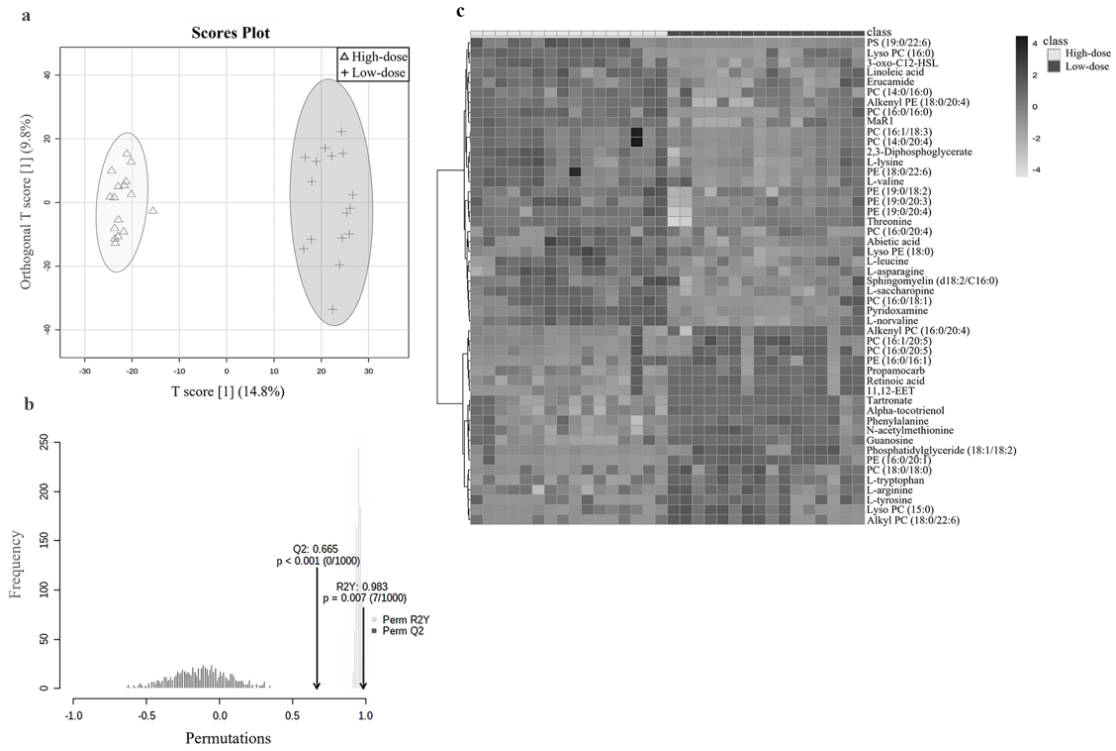

Figure 4. Metabolomic alteration between the low-dose and high-dose groups. (a) The metabolites in the low-dose and high-dose groups were clearly separated by the orthogonal partial least squares discrimination analysis model. (b) The model had no evidence of overfitting. One thousand permutation tests yielded an R^2Y value of 0.983 (p = 0.007) and a Q^2 value of 0.665 (p<0.001). (c) Heat map of differential metabolites in the high-dose group compared with the low-dose group. The shades of the color represented metabolites levels (black, and white indicated higher level, and lower level, respectively).

The current results are consistent with those of studies that have reported that probiotic supplementation can lower the concentration of N-acetylmethionine,[20] a methyl donor.[21] Decreased N-acetylmethionine diminishes the methylation of insulin-like growth factor binding protein 1 and methionine sulfoxide reductase A and reduces the risk of aberrant glucose metabolism.[20] Moreover, studies have indicated that MaR1 can induce bactericidal/permeability-increasing protein expression and Nrf2 nuclear translocation.[22] Therefore, MaR1 can improve the anti-inflammatory and antimicrobial properties of *M. tuberculosis*-infected human macrophages.[23] MaR1 can also reduce *M. tuberculosis*-induced TNF-α production.[22] Additionally, increased indoleamine 2,3 dioxygenase 1 (IDO-1)–mediated tryptophan catabolism may modulate the CD4+ T cell responses of patients with TB, alleviating inflammation and inducing immune tolerance.[24] However, daily probiotic supplementation can limit the drops in tryptophan concentrations,[25] and thus affect body immunity, likely by increasing metabolites.

In patients with TB, the concentrations of IFN-γ, TNF-α, IL-10, and IL-12 may increase.[26,27] In the present trial, *L. casei* supplementation led to a significant reduction of TNF-α, IL-6, IL-10, and IL-12 concentrations. Studies have consistently reported that *L. casei* supplementation lowered TNF-α, IL-6, and IL-12 concentrations.[28,29] The IL-10 concentration also decreased in this study, possibly because of the immune-stimulatory effects of IL-10. IL-10 exerts these effects by inducing bal-2 protein expression to inhibit peripheral T cell apoptosis or by promoting the proliferation and differentiation of B lymphocytes into plasmocytes.[30,31]

Correlation analysis may explain the regulating effect of metabolites on inflammatory cytokines. Studies have demonstrated that PS treatment reduces the concentrations of TNF-α and IL-6.[32,33] Apoptosis occurs during the fight against *M. tuberculosis*, and PS inhibits the phagocytosis of apoptotic cells and induces an anti-inflammatory state.[34] This study indicated a strong correlation between PS (19:0/22:6) and IL-12, likely because IL-12 is anti-inflammatory. Tryptophan can be catabolized by IDO-1 in splenic macrophages. Vitro experiments displayed that expression of IDO-1 significantly suppresses IL-12 production in splenic macrophages through a primary downstream effector, metabolic-stress sensing protein kinase General Control Non-depressible 2.[35] PC is the precursor of lysophosphatidylcholine, a chemoattractant for T lymphocytes,[36] and PC lowered the concentrations of TNF-α and IL-6 in rat experiments.[37,38] Overall, the present results indicated that *L. casei* may regulate the plasma metabolic profile and inflammatory cytokine concentrations of patients with TB, and inflammatory cytokine changes may partly cause the changes in the metabolites.

This study has several strengths. First, this is the first clinical trial to investigate the effect of probiotics on plasma inflammatory cytokines and metabolites in patients with TB, and the results indicated that *L. casei* supplementation modulates inflammatory cytokines and metabolites in patients with TB. Second, the potential correlations between the abundance of plasma metabolites and the concentrations of inflammatory cytokines were examined. The effect of *L. casei* supplementation on inflammatory cytokines may be related to the metabolite changes.

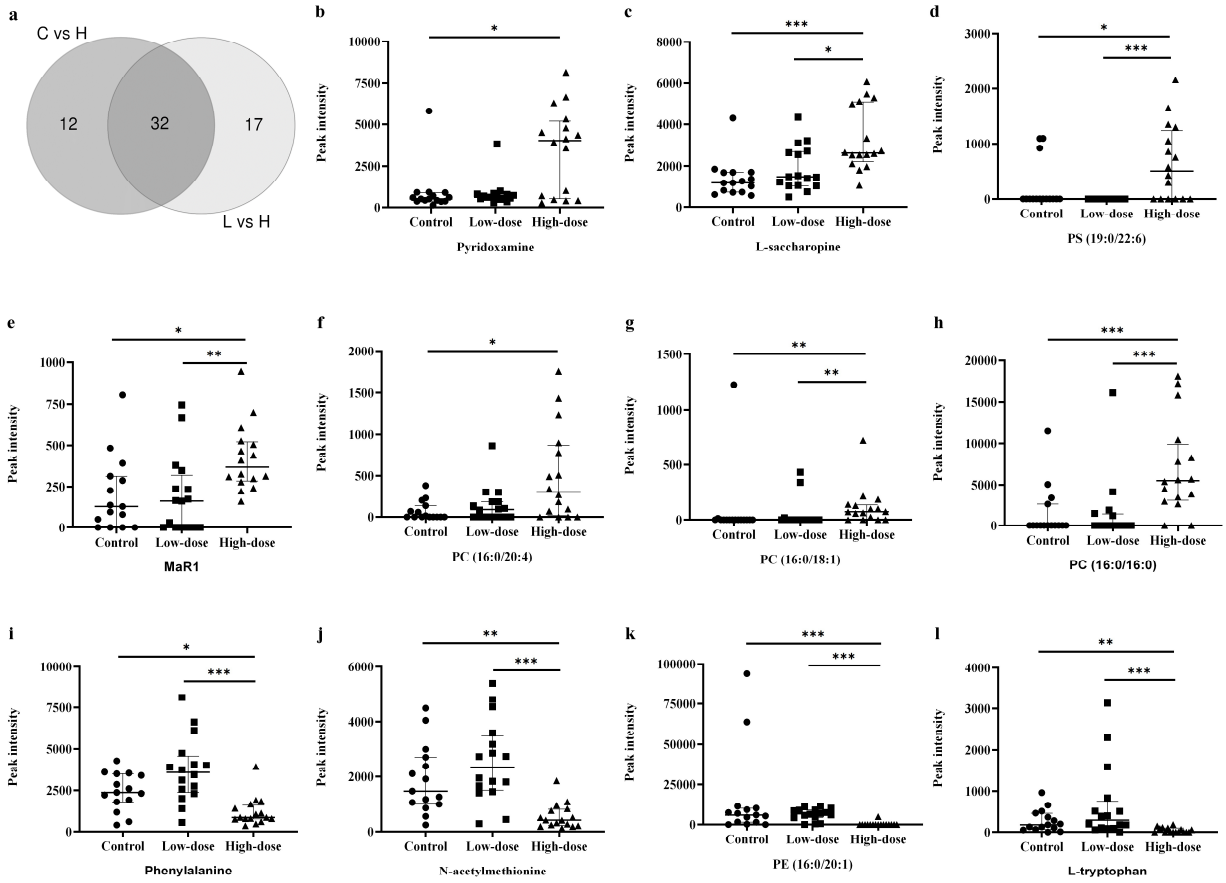

Figure 5. (a) Venn diagram displayed the number of differential metabolites in the control group vs high-dose group, low-dose group vs high-dose group. The values for shared differential metabolites: (b-l) Peak intensity of 11 differential metabolites in the control, low-dose and high-dose groups. Data were displayed as scatter plots with median and interquartile range, with each dot representing one individual. Peak intensities of metabolites were analyzed with a nonparametric test with a Dunn's multiple comparisons test conducted between groups. *p<0.05, **p<0.01, ***p<0.001.

Third, patients with TB were all inpatients during the first 4 weeks of supplementation and shared similar living and dietary habits, which could have increased the authenticity of the results.

Limitations of the study should also be acknowledged. The follow-up duration of 1 month prevented an investigation on the long-term effects of *L. casei* during TB treatment on patients' metabolic profiles and immunity conditions, which had been altered when patients were infected with *M. tuberculosis*. The results demonstrated that a 1-month intervention resulted in significant improvements. Second, the present study only employed two supplementation dosages; therefore, elucidating the dosage of *L. casei* supplementation for modulating metabolites and the concentrations of inflammatory cytokines is difficult.

In conclusion, daily *L. casei* supplementation of up to 2 × 10^{10} CFU during the intensive phase of TB treatment modulates metabolites and inflammatory cytokines. Decreased concentrations of inflammatory cytokines may be related to the metabolite changes. Future work can investigate the effect of long-term probiotic interventions in patients with TB.

AUTHOR DISCLOSURES

The authors reported no conflict of interest. The National Natural Science Foundation of China (No. 81673160 and No. 81803222) supported the research.

Yakult Honsha Co., Ltd provided *L. casei* preparation used in this study in sponsorship character. The funders did not exert any influence on study design, data collection, data analysis and manuscript preparation.

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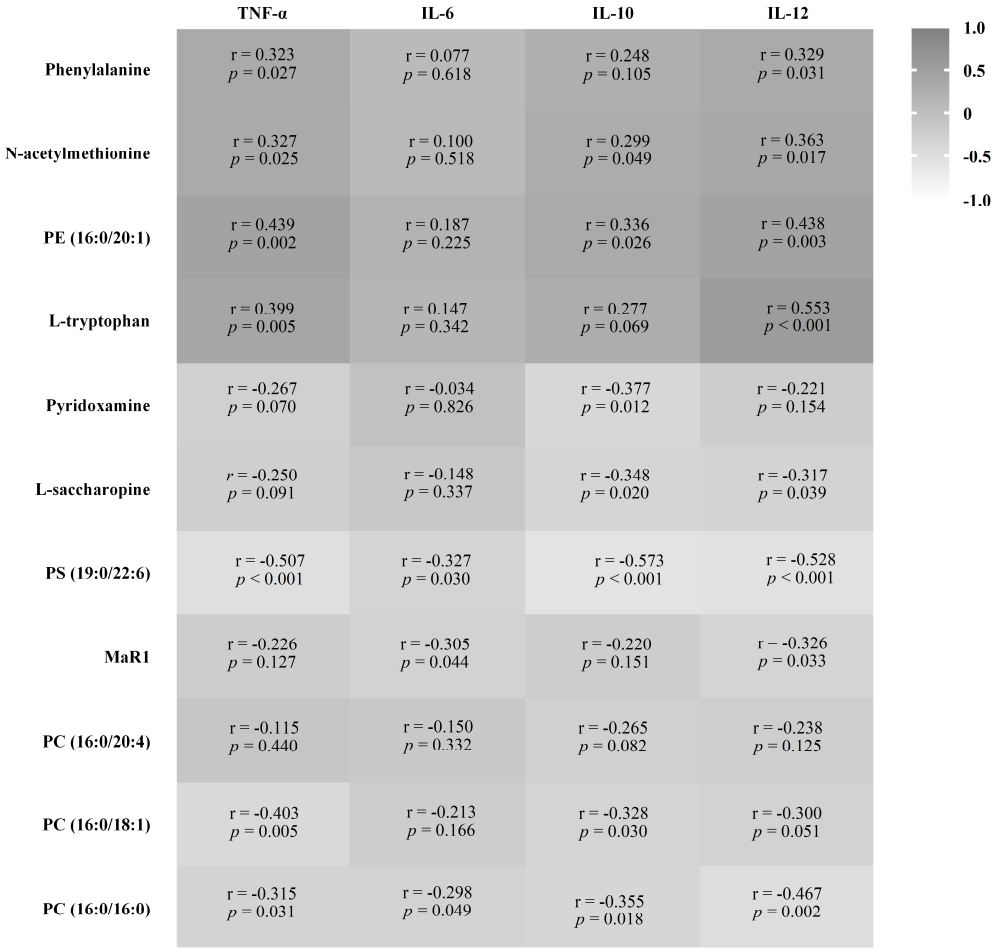

Figure 6. Correlations between inflammatory cytokines and metabolites through Spearman nonparametric test. The shades of the color represented correlations levels (Black, and white indicated positive correlation, and negative correlation, respectively).

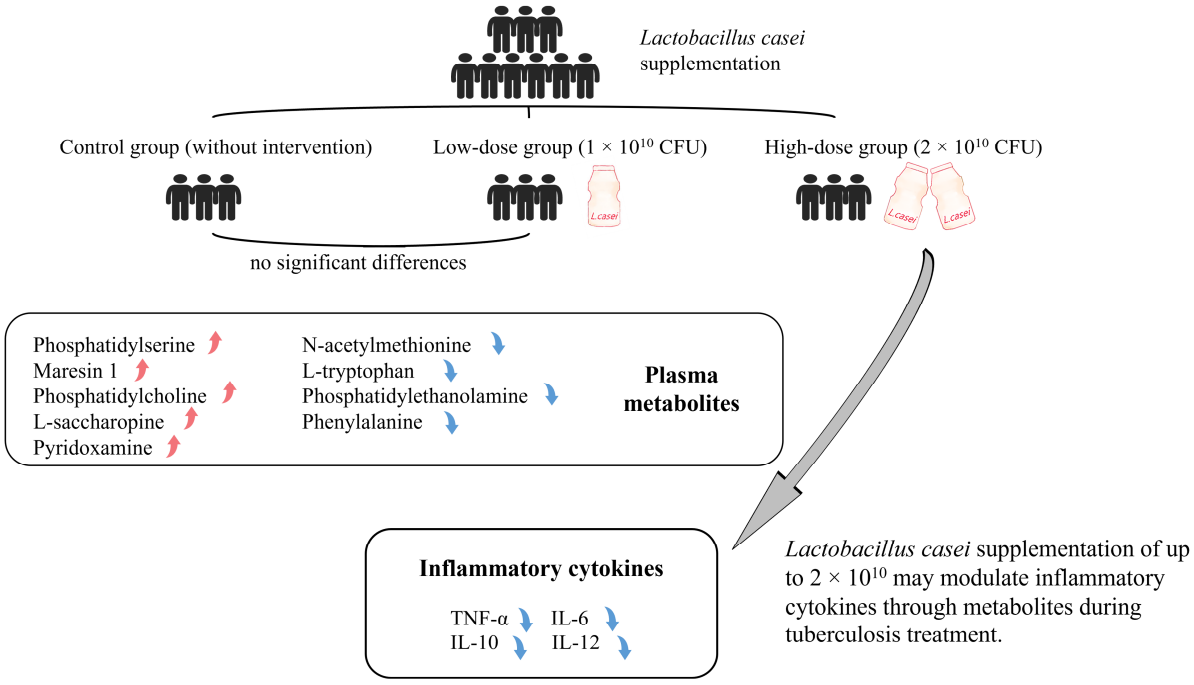

Figure 7. Graphical abstract.

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Supplementary table 1. The differential metabolites between the control and high-dose groups in plasma

Metabolite name	FC	log2(FC)	*p*. adjusted
Phosphatidylethanolamine (18:0/20:3)	0.01	-6.72	2.84E-02
Phosphatidylethanolamine alkenyl (18:0/18:1)	0.01	-6.19	2.20E-02
Phosphatidylethanolamine (16:0/20:1)	0.04	-4.65	4.82E-04
Phosphatidylcholine alkyl (18:0/22:6)	0.05	-4.35	3.73E-02
Phosphatidylglyceride (18:1/18:2)	0.10	-3.37	2.90E-02
Phosphatidylcholine lyso (15:0)	0.11	-3.17	3.77E-02
Hyperoside	0.15	-2.75	2.97E-02
gamma-tocotrienol	0.22	-2.17	3.35E-02
alpha-tocotrienol	0.23	-2.10	9.52E-03
Retinoic acid	0.24	-2.05	9.76E-03
Tartronate	0.27	-1.88	7.09E-03
L-tryptophan	0.27	-1.87	9.92E-03
1-nitropyrene	0.29	-1.81	2.56E-02
Phosphatidylcholine (16:1/20:5)	0.29	-1.78	2.72E-02
Phosphatidylcholine alkenyl (16:0/20:4)	0.31	-1.69	6.81E-03
11, 12 epoxyeicosatrienoic acid	0.33	-1.61	2.93E-03
Guanosine	0.33	-1.58	1.18E-02
Phosphatidylcholine (16:0/20:5)	0.40	-1.34	3.09E-02
N-acetylmethionine	0.41	-1.28	7.34E-03
Propamocarb	0.46	-1.11	9.40E-03
Phosphatidylethanolamine (19:0/18:2)	0.50	-1.00	2.27E-02
Phenylalanine	0.65	-0.61	3.73E-02
L-valine	1.37	0.46	4.65E-02
L-norvaline	1.60	0.68	7.75E-03
Erucamide	1.70	0.77	3.51E-02
Phosphatidylcholine (16:0/18:1)	1.80	0.84	8.27E-03
Threonine	1.85	0.89	4.06E-02
Sphingomyelin (d18:2/C18:0)	1.94	0.95	3.53E-02
D-ribose	1.94	0.96	1.08E-02
Maresin 1	2.04	1.03	1.28E-02
L-asparagine	2.12	1.08	9.55E-03
Phosphatidylserine (19:0/22:6)	2.55	1.35	4.13E-02
Phosphatidylethanolamine lyso (18:0)	2.62	1.39	9.92E-03
Phosphatidylcholine (14:0/16:0)	2.87	1.52	1.45E-02
L-saccharopine	2.90	1.54	1.34E-04
Phosphatidylethanolamine alkenyl (17:0/22:4)	3.43	1.78	3.02E-02
Triacylglycerol (10:0/18:1/18:2)	3.44	1.78	3.20E-02
N-3-oxo-dodecanoyl-L-Homoserine lactone	3.55	1.83	1.51E-02
Phosphatidylcholine (17:0/18:2)	3.60	1.85	1.24E-02
Phosphatidylcholine (16:0/16:0)	3.73	1.90	5.15E-03
Histidine	4.09	2.03	7.29E-03
Phosphatidylcholine (19:0/22:6)	4.10	2.04	4.49E-02
Pyridoxamine	4.28	2.10	2.99E-03
Phosphatidylcholine (16:0/20:4)	9.17	3.20	2.52E-02

Supplementary table 2. The differential metabolites between the high-dose and low-dose groups in plasma

Metabolite name	FC	log2(FC)	*p*. adjusted
Phosphatidylcholine alkyl (18:0/22:6)	0.04	-4.77	1.96E-02
Phosphatidylcholine lyso (15:0)	0.12	-3.10	2.68E-02
Phosphatidylglyceride (18:1/18:2)	0.13	-2.99	7.78E-03
L-tryptophan	0.15	-2.77	3.20E-03
Phosphatidylethanolamine (16:0/20:1)	0.15	-2.75	1.35E-05
Phosphatidylcholine (18:0/18:0)	0.16	-2.67	3.03E-02
Tartronate	0.22	-2.16	5.96E-04
Retinoic acid	0.25	-2.02	7.04E-04
alpha-tocotrienol	0.25	-1.98	1.17E-03
L-tyrosine	0.29	-1.79	4.46E-02
11, 12 epoxyeicosatrienoic acid	0.31	-1.69	1.87E-04
Phosphatidylcholine alkenyl (16:0/20:4)	0.33	-1.62	1.66E-02
Phosphatidylcholine (16:1/20:5)	0.34	-1.58	3.01E-02
L-arginine	0.34	-1.56	2.35E-02
Guanosine	0.34	-1.54	6.50E-04
N-acetylmethionine	0.37	-1.42	1.52E-03
Propamocarb	0.38	-1.39	7.28E-03
Phosphatidylethanolamine (16:0/16:1)	0.40	-1.33	4.23E-02
Phosphatidylcholine (16:0/20:5)	0.41	-1.28	1.30E-02
Phenylalanine	0.53	-0.91	3.32E-03
L-valine	1.45	0.54	9.68E-03
Abietic acid	1.59	0.66	1.25E-02
Erucamide	1.59	0.67	3.02E-02
Linoleic acid	1.63	0.71	1.16E-02
Threonine	1.68	0.75	1.89E-02
L-leucine	1.77	0.82	4.75E-03
L-asparagine	1.77	0.83	8.53E-03
Phosphatidylcholine (14:0/16:0)	1.79	0.84	2.25E-02
Phosphatidylcholine (14:0/20:4)	1.80	0.85	5.06E-03
Phosphatidylcholine (16:1/18:3)	1.80	0.85	5.06E-03
L-lysine	1.81	0.85	2.75E-02
Phosphatidylcholine (16:0/18:1)	1.89	0.91	5.47E-03
L-norvaline	1.91	0.94	4.48E-05
Phosphatidylethanolamine (18:0/22:6)	1.95	0.96	1.25E-02
2,3-Diphosphoglycerate	2.01	1.01	2.02E-03
Phosphatidylethanolamine alkenyl (18:0/20:4)	2.15	1.10	1.60E-02
Phosphatidylethanolamine lyso (18:0)	2.36	1.24	1.16E-02
Sphingomyelin (d18:2/C16:0)	2.43	1.28	4.95E-02
L-saccharopine	2.66	1.41	6.31E-05
Maresin 1	2.95	1.56	2.53E-03
Phosphatidylethanolamine (19:0/20:4)	3.08	1.62	3.84E-02
Phosphatidylcholine lyso (16:0)	3.50	1.81	1.49E-03
Phosphatidylethanolamine (19:0/20:3)	3.64	1.86	1.77E-02
Phosphatidylcholine (16:0/16:0)	4.74	2.25	5.96E-04
Pyridoxamine	4.75	2.25	4.31E-04
Phosphatidylcholine (16:0/20:4)	5.23	2.39	2.51E-02
Phosphatidylethanolamine (19:0/18:2)	5.38	2.43	2.54E-02
N-3-oxo-dodecanoyl-L-Homoserine lactone	5.70	2.51	6.67E-04
Phosphatidylserine (19:0/22:6)	14.39	3.85	9.68E-05

Original Article

Perioperative enteral immunonutrition with probiotics favors the nutritional, inflammatory, and functional statuses in digestive system surgery

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Background and Objectives: This study aimed to evaluate the effects of enteral immunonutrition (EIN) on the nutritional status of patients during the perioperative period of digestive system surgery. **Methods and Study Design:** The clinical data of 102 patients who underwent gastrointestinal surgery between August 2017 and February 2021 were retrospectively analyzed. According to the nutritional support regimen, the patients were divided into an enteral nutrition (EN) group (50 patients) and an EIN group (52 patients). **Results:** The times (in hours) to return of the first bowel sound, first postoperative flatus, and first bowel movement, as well as the length of postoperative hospital stay were shorter in the EIN group than in the EN group (p<0.05). The concentrations of hemoglobin, prealbumin, albumin, and transferrin, as well as the concentrations of immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), complement C3, and complement C4 were higher in the EIN group than in the EN group at 1 and 7 days after surgery (p<0.05). The concentrations of endotoxins, D-lactic acid, and diamine oxidase were lower in the EIN group than in the EN group (p<0.05). The tolerance to enteral feeding was better in the EIN group than in the EN group (p<0.05). The incidence of complications was lower in the EIN group (5.77%) than in the EN group (10.0%) (p>0.05). **Conclusions:** EIN can promote gastrointestinal function recovery, improve the nutritional status, enhance the humoral immune function, regulate intestinal flora balance, improve intestinal permeability, prevent enteral feeding intolerance, and reduce complications in patients undergoing surgery for digestive system diseases.

Key Words: digestive system surgery, microbial immune enteral nutrition, nutritional status, immune function, intestinal flora

INTRODUCTION

Patients often experience varying degrees of immunosuppression and malnutrition due to reduced nutrient intake, abnormal catabolism, absorption and digestive dysfunctions, and changes in the anatomical and physiological structures of the digestive tract. Moreover, surgical stress can cause bacterial/endotoxin translocation and intestinal mucosal atrophy, further aggravating the dysfunction of the immune system.[1-3] Therefore, providing timely immune regulation and nutritional support is particularly crucial in improving the nutritional status of patients during the perioperative period, as well as in reducing complications and accelerating recovery.

Parenteral nutrition is a traditional mode of nutritional support after digestive system surgeries. Although it can meet the nutritional needs of patients, long-term parenteral nutrition can destroy the intestinal mucosal barrier because of the lack of food in the intestines, leading to intestinal mucosal atrophy and inflammation in the body.[4,5] Enteral nutrition (EN) can maintain the integrity of the intestinal mucosa, protect the intestinal barrier function, increase visceral blood flow, promote the normal growth of the intestinal flora, and enhance the function of gut-associated lymphoid tissues.[6] Meanwhile, enteral immunonutrition (EIN) can regulate the activity of the immune system during the perioperative period. Microbial EIN combines an enteral nutrient solution with probiotics, arginine, glutamate, and other nutritional components to regulate the intestinal flora. As a result, it can enhance the immune response, regulate the release and production of cytokines, inhibit intestinal flora imbalance, maintain growth, and reduce systemic inflammation.[7,8] A meta-analysis found that EIN can regulate the inflammation level, improve the cellular immune function, and reduce postoperative complications in patients undergoing radical gastrointestinal cancer surgery.[9] An animal experiment study reported that it can effectively inhibit the inflammatory response in rats with acute pancreatitis, and its mechanism of action is related to the regulation of the Janus kinase 2/signal transducer and activator of transcription 3 signaling pathway.[10] This study aimed to

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investigate the effects of EIN on the nutritional status, immune function, intestinal flora, and intestinal permeability of patients during the perioperative period of digestive system surgery.

METHODS

Clinical data

The clinical data of 102 patients who underwent gastrointestinal surgery at our hospital between August 2017 and February 2021 were retrospectively analyzed. The patients were divided into an EN group (50 patients) and an EIN group (52 patients) according to the specific nutritional support regimen. The EN group consisted of 29 male and 21 female patients with an average age of 53.7±8.12 years (range, 28–76 years) and an average body mass index of 21.5±2.58 kg/m^2. Their diagnoses were as follows: cancer (n=31) (esophageal cancer, n=8; gastric cancer, n=9; colorectal cancer, n=8; and bladder cancer, n=6) and benign lesions (n=19) (intestinal perforation, n=6; intestinal obstruction, n=9; esophageal stenosis, n=3; and intestinal necrosis, n=1); educational level: junior middle school or below (n=18), senior high school or technical secondary school (n=20), and junior college or above (n=12); residence: urban areas (n=38) and rural areas (n=12); payment methods for medical expenses: medical insurance (n=35), new rural cooperative medical care system (n=10), and own expense (n=5); and underlying disease: hypertension (n=10), coronary heart disease (n=8), diabetes (n=7), stroke (n=2), and fatty liver (n=2). Their average preoperative Nutritional Risk Screening 2002 (NRS-2002) score was 4.56±0.52 points; hemoglobin (Hb) concentration, 126±13.2 g/L; prealbumin (PA) concentration, 266±28.2 mg/L; albumin (ALB) concentration, 35.3±3.02 g/L; and transferrin (TRF) concentration, 2.46±0.29 g/L on admission. Meanwhile, the EIN group consisted of 30 male and 22 female patients with an average age of 54.1±7.46 years (range, 25–75 years) and an average body mass index of 22.0±2.49 kg/m^2. Their diagnoses were as follows: cancer (n=28) (esophageal cancer, n=7; gastric cancer, n=7; colorectal cancer, n=9; and bladder cancer, n=5) and benign lesions (n=24) (intestinal perforation, n=8; intestinal obstruction, n=10; esophageal stenosis, n=4; and intestinal necrosis, n=2); educational level: junior middle school or below (n=17), senior high school or technical secondary school (n=21), and junior college or above (n=14); residence: urban areas (n=37) and rural areas (n=15); payment methods for medical expenses: medical insurance (n=33), new rural cooperative medical care system (n=9), and own expense (n=10); and underlying disease: hypertension (n=9), coronary heart disease (n=7), diabetes (n=8), stroke (n=3), and fatty liver (n=1). Their preoperative NRS-2002 score was 4.63±0.38 points; Hb concentration, 126±13.0 g/L; PA concentration, 266±27.2 mg/L; ALB concentration, 36.2±2.97 g/L; and TRF concentration, 2.51±0.33 g/L on admission. The types of surgery in the EIN group were as follows: partial gastrectomy or subtotal gastrectomy (n=12), esophagectomy (n=14), partial colectomy or colon segmental resection (n=11), pancreaticoduodenectomy (n=11), and other surgeries (n=4). The general data of the patients were comparable between the EIN and EN groups (p>0.05). The study design and conceptual framework are shown in Figures 1 and 2.

Selection criteria

The inclusion criteria were as follows: preoperative NRS-2002 score of ≥3 points, age of ≥18 years, indications for nutritional support, clear consciousness and no communication or mental disorders, and voluntarily signed informed consent form. The exclusion criteria were as follows: preoperative infection; requirement for emergency surgery; comorbidities, including Addison's disease, hyperthyroidism, or hypothyroidism; radiotherapy and chemotherapy upon admission; surgery time of >6 h and intraoperative blood loss amount of >500 mL; use of immunosuppressant and glucocorticoid therapy; immune system disease; and severe heart, lung, kidney, and liver insufficiencies before surgery. This study was approved by the Ethics Committee of The Ninth People's Hospital of Chongqing.

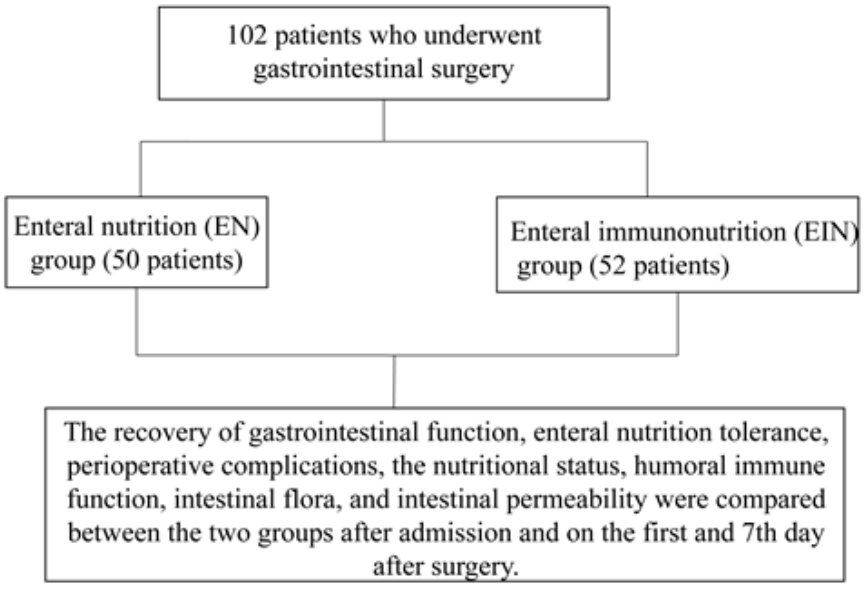

Figure 1. Study design of the study.

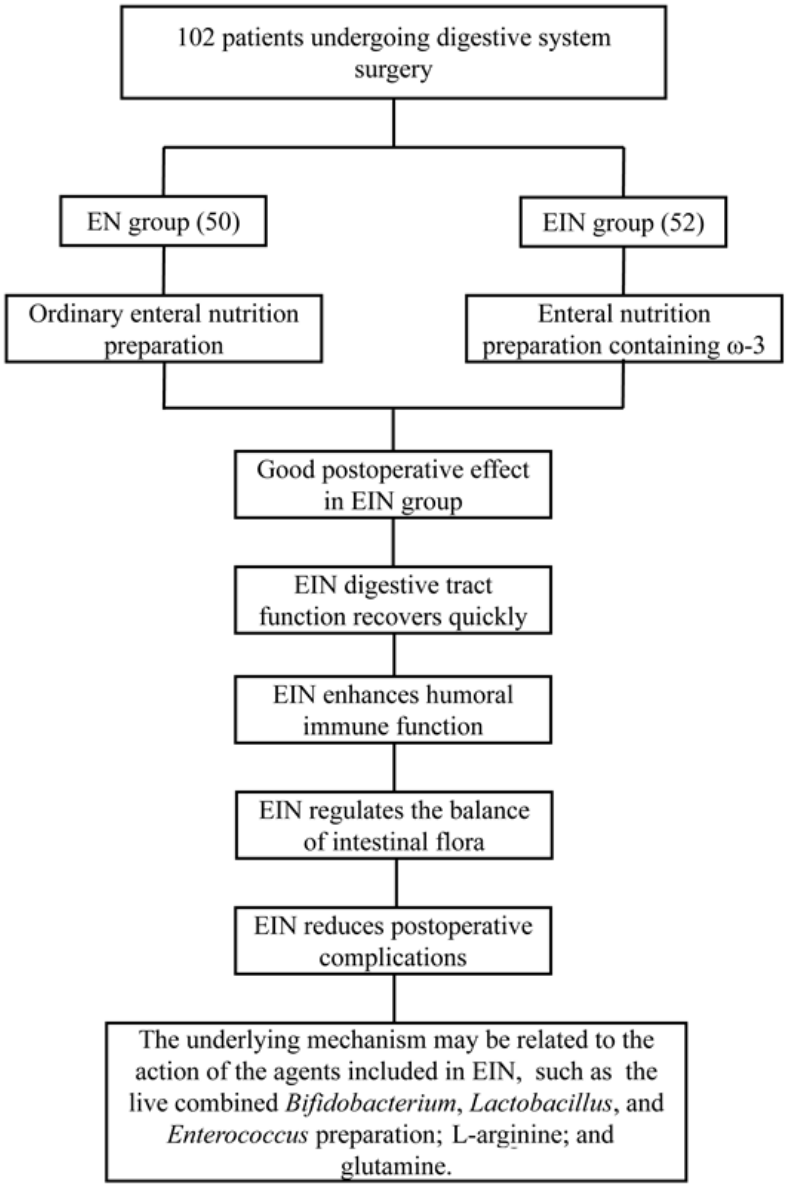

Figure 2. Conceptual framework of the study.

Methods

All patients were provided with 25 kcal/kg EN 3 days before surgery, and the intervention was discontinued 6–8 h before surgery. After 6 h, the stability of the patients' vital signs was checked, and EN was administered via a gastric tube. The EIN group was administered an EN preparation containing omega-3 fatty acids (Ruidai, Huarui Pharmaceutical Co., Ltd.). In addition, 6 g/day live combined *Bifidobacterium*, *Lactobacillus*, and *Enterococcus* preparation; 0.25 g/(kg·day) L-arginine; and 0.4 g/(kg·day) glutamate were administered three times a day. The EN group received common enteral nutrient preparations (Ruidai, Huarui Pharmaceutical Co., Ltd.).

Outcome measurements

1. Gastrointestinal function recovery. The times (in hours) to the first postoperative flatus and first bowel movement, and the length of postoperative hospital stay were recorded.
2. Sample collection. Fasting cubital venous blood (5 mL) was collected from all patients after admission and at 1 and 7 days after surgery. The blood samples were allowed to stand at room temperature for 30 min and centrifuged thereafter for 10 min (R=6 cm, 3500 r/min). The serum was separated and refrigerated at -80℃.
3. Nutritional status. The serum Hb, PA, ALB, and TRF concentrations were measured using an automatic biochemical analyzer (Atellica CH930, Shanghai Jumu Medical Instruments Co., Ltd.).
4. Humoral immune function. The serum immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), complement C3, and complement C4 concentrations were determined using an enzyme-linked immunosorbent assay kit (Guangdong Gukang Biotechnology Co., Ltd.).
5. Intestinal flora. After admission and at 1 and 7 days after surgery, 5–8 g fresh stool specimens were collected and cultured for bacteria. *Lactobacillus*, *Bifidobacterium*, *Escherichia coli* (*E. coli*), *Staphylococcus*, and *Enterococcus faecalis* were enumerated using the plate count technique.
6. Intestinal permeability. Enzyme-linked immunosorbent assay was used to determine the endotoxin

concentrations; enzymatic spectrophotometry (Shanghai Fantai Biotechnology Co., Ltd.) was used to measure the dextro-lactate dehydrogenase concentrations; and colorimetry (Amictech Co., Ltd.) was used to determine the diamine oxidase (DAO) concentrations.

7. Enteral feeding tolerance. Patients who were able to tolerate the target enteral nutrient solution, without obvious unfavorable reactions, were considered to have complete tolerance. Patients who experienced mild adverse reactions and received more than one-half of the target volume of the enteral nutrient solution after slowing down the administration were determined to have partial tolerance. Patients who developed abdominal pain, abdominal distension, and other adverse reactions; received less than one-half of the target volume of the enteral nutrient solution; had watery stool that occurred more than four times within 24 h; or developed a sore throat were considered to have intolerance to enteral feeding.
8. Complications. The occurrence of deep vein thrombosis, incisional infection, abdominal cavity infection, urinary tract infection, anastomotic leakage, reflux pneumonia, and other complications was recorded.

Statistical analysis

The SPSS software (version 24.0) was used for all statistical analyses. Measurement data were reported as means ± standard deviations (means ± SDs). The independent t-test and paired t-test were used for comparisons between the groups. Count data were expressed as rates and examined using the chi-square test. Statistical significance was set at $p<0.05$.

RESULTS

Gastrointestinal function recovery

The times (in hours) to return of the first bowel sound, first postoperative flatus, and first bowel movement, as well as the length of postoperative hospital stay were shorter in the EIN group than in the EN group ($p<0.05$); this indicates that EIN could promote an earlier recovery of the gastrointestinal function after surgery in patients with digestive system diseases (Table 1).

Nutritional status

The concentrations of the nutritional indices after admission were not significantly different between the two groups ($p>0.05$). However, the concentrations of Hb, PA, ALB, and TRF were higher in the EIN group than in the EN group at 1 and 7 days after surgery ($p<0.05$), indicating that EIN could improve the perioperative nutritional status of patients with digestive system diseases (Figure 3).

Humoral immune function

The concentrations of the humoral immune indices after admission did not significantly differ between the EIN and EN groups ($p>0.05$). However, the concentrations of IgA, IgG, IgM, complement C3, and complement C4 were higher in the EIN group than in the EN group at 1 and 7 days after surgery ($p<0.05$), indicating that EIN could enhance the postoperative humoral immune function of patients with digestive system diseases (Figure 4).

Intestinal flora

The intestinal flora indices after admission were not significantly different between the EIN and EN groups ($p>0.05$). However, the *Lactobacillus* and *Bifidobacterium* counts were higher in the EIN group than in the EN group at 1 and 7 days after surgery, whereas the *E. coli*, *Staphylococcus*, and *Faecococcus* counts were lower in the EIN group than in the EN group ($p<0.05$); this indicates that EIN could regulate the balance of the intestinal flora, supplement beneficial bacteria, and inhibit the growth of pathogenic bacteria after surgery in patients with digestive system diseases (Table 2).

Intestinal permeability

The concentrations of the intestinal permeability indices after admission were not significantly different between the two groups ($p>0.05$). However, the endotoxin, D-lactate, and DAO concentrations were lower in the EIN group than in the EN group at 1 and 7 days after surgery ($p<0.05$), indicating that EIN could improve intestinal permeability in patients with digestive system diseases (Table 3).

Enteral feeding tolerance

The enteral feeding tolerance was better in the EIN group than in the EN group ($p<0.05$), indicating that EIN could improve the enteral feeding tolerance in patients with digestive system diseases (Table 4).

Complications

Although the difference was not significant, the incidence of complications was lower in the EIN group (5.77%) than in the EN group (10.0%) ($p>0.05$), indicating that EIN is a safer regimen than EN (Table 5).

Table 1. Comparison of the gastrointestinal function

	EN group (n=50)	EIN group (n=52)
Time to return of the first bowel sound (h)	40.2±6.12	35.9±5.12***
Time to the first postoperative flatus (h)	54.1±7.25	45.8±6.25***
Time to the first bowel movement (h)	65.3±6.25	58.8±4.16***
Duration of postoperative hospital stay (d)	11.0±2.64	8.31±2.46***

EN: enteral nutrition; EIN: enteral immunonutrition.
Data are presented as means ± SDs.
***$p<0.001$ vs the EN group.

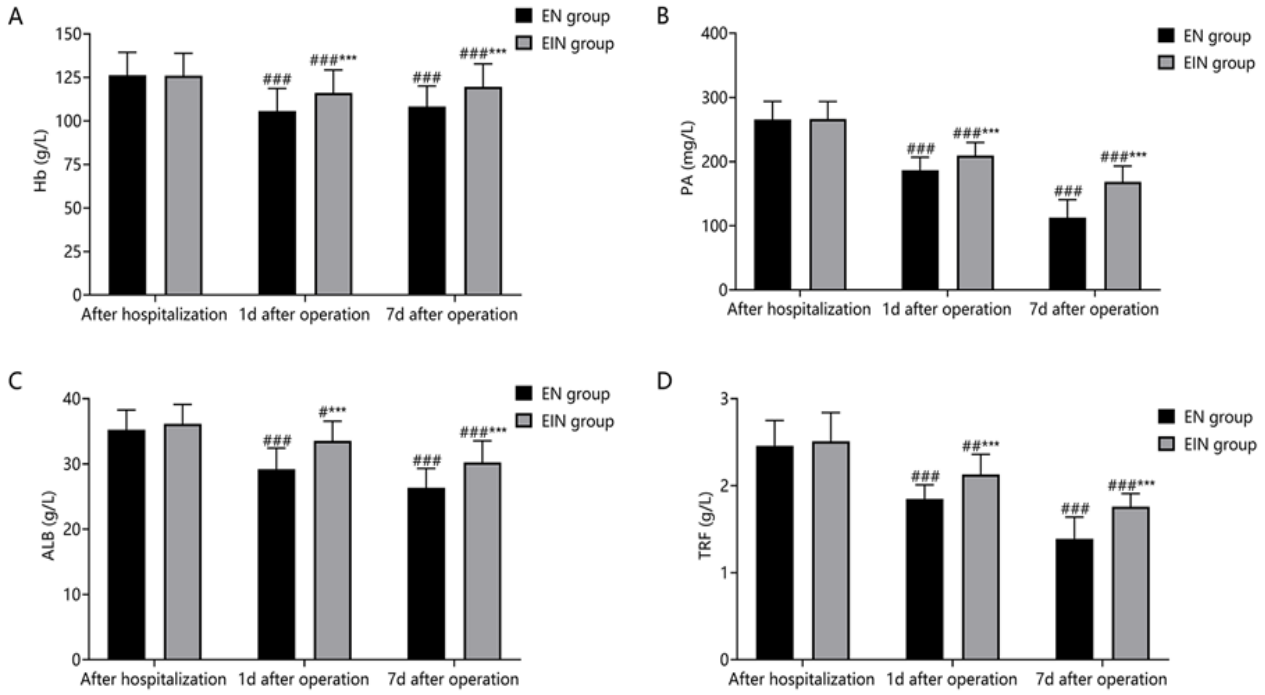

Figure 3. Comparison of the nutritional indicators between the two groups. (A) The Hb concentrations were significantly higher in the EIN group than in the EN group at 1 and 7 days postoperatively. (B) The PA concentrations were significantly higher in the EIN group than in the EN group at 1 and 7 days postoperatively. (C)The ALB concentrations were significantly higher in the EIN group than in the EN group at 1 and 7 days postoperatively. (D) The TRF concentrations were significantly higher in the EIN group than in the EN group at 1 and 7 days postoperatively. $^{\#}p<0.05$, $^{\#\#}p<0.01$, and $^{\#\#\#}p<0.001$ vs after admission; $^{***}p<0.001$ vs. the EN group; Hb: hemoglobin; EIN: enteral immunonutrition; EN: enteral nutrition; PA: prealbumin; ALB: albumin; TRF: transferrin.

Table 2. Comparison of the gastrointestinal function

	EN group (n=50)	EIN group (n=52)
Lactobacillus		
After admission	8.03±0.46	7.96±0.52
1 d postoperatively	8.06±0.43	8.68±0.56$^{\#*}$
7 d postoperatively	7.56±0.39	9.67±0.48$^{\#\#\#***}$
Bifidobacterium		
After admission	7.16±0.65	7.11±0.64
1 d postoperatively	7.20±0.56	7.95±0.55$^{\#***}$
7 d postoperatively	6.95±0.37	8.92±0.66$^{\#\#\#***}$
Escherichia coli		
After admission	7.59±0.46	7.82±0.55
1 d postoperatively	7.62±0.52	6.85±0.32$^{\#**}$
7 d postoperatively	8.02±0.51	6.38±0.38$^{\#\#***}$
Staphylococcus		
After admission	4.12±0.49	4.28±0.38
1 d postoperatively	4.13±0.51	3.49±0.52$^{\#**}$
7 d postoperatively	4.08±10.5	3.32±0.38$^{\#\#***}$
Enterococcus faecalis		
After admission	7.63±0.86	7.82±0.89
1 d postoperatively	7.56±0.56	6.89±0.35$^{\#\#**}$
7 d postoperatively	7.69±0.67	6.68±0.39$^{\#\#\#***}$

EN: enteral nutrition; EIN: enteral immunonutrition.
Data are presented as means ± SDs in log colony-forming units/g.
$^{\#}p<0.05$, $^{\#\#}p<0.01$, and $^{\#\#\#}p<0.001$ vs after admission.
$^{**}p<0.01$ and $^{***}p<0.001$ vs the EN group.

DISCUSSION

The metabolic response to stress induced by surgical trauma can alter the Ig synthesis and intestinal mucosal barrier function and damage immune cells. An impaired intestinal mucosal barrier can lead to an uncontrolled release of inflammatory cytokines and excessive production of inflammatory mediators, further disrupting the ecological balance of the intestinal flora and the immune homeostasis and increasing the permeability of the intestinal mucosal barrier.[11, 12] Enteral microecological nutrition can balance the intestinal flora, and EN can enhance the immune function. Meanwhile, EIN can provide both benefits through its synergistic effects.[13]

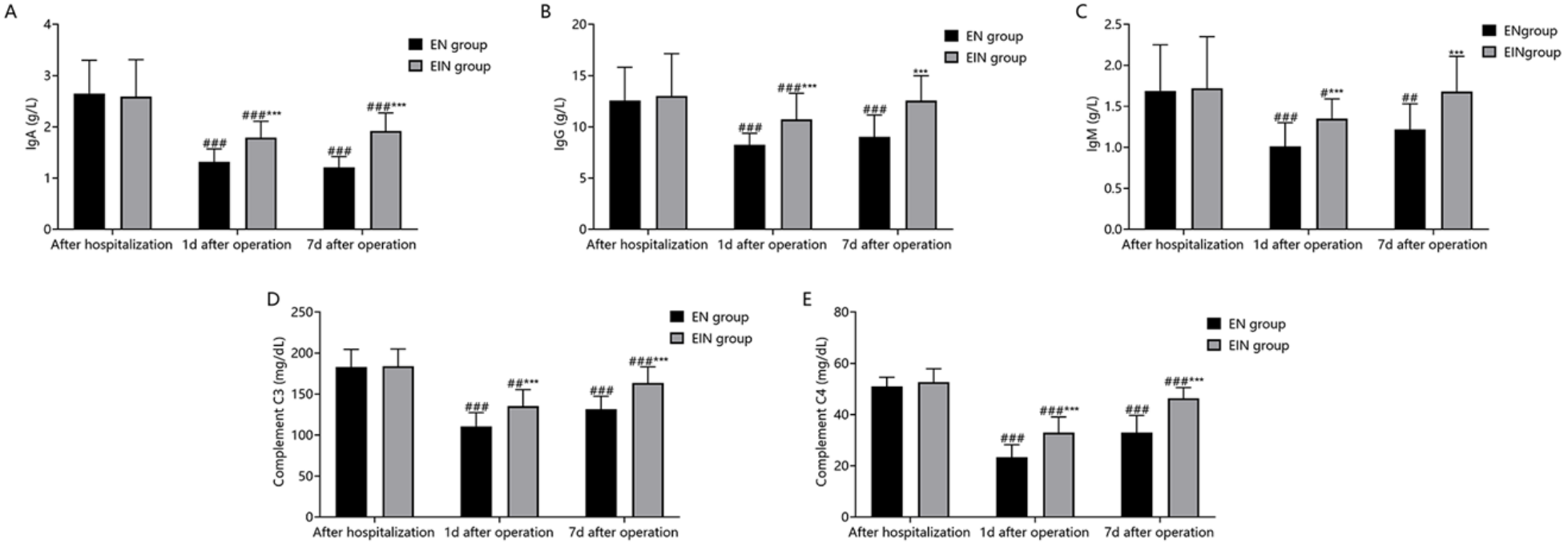

Figure 4. Comparison of the humoral immune function between the two groups. (A) The IgA concentrations were significantly higher in the EIN group than in the EN group at 1 and 7 days postoperatively. (B) The IgG concentrations were significantly higher in the EIN group than in the EN group at 1 and 7 days postoperatively. (C) The IgM concentrations were significantly higher in the EIN group than in the EN group at 1 and 7 days postoperatively. (D) The complement C3 concentrations were significantly higher in the EIN group than in the EN group at 1 and 7 days postoperatively. (E) The complement C4 concentrations were significantly higher in the EIN group than in the EN group at 1 and 7 days postoperatively. $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ vs. after admission; $^{***}p<0.001$ vs the EN group; Ig: immunoglobulin; EIN: enteral immunonutrition; EN: enteral nutrition.

Table 3. Comparison of the intestinal permeability

	EN group (n=50)	EIN group (n=52)
Endotoxin (pg/mL)		
After admission	1.52±0.31	1.29±0.29
1 d postoperatively	2.97±0.43$^{\#\#\#}$	2.02±0.37$^{\#\#\#**}$
7 d postoperatively	2.88±0.52$^{\#\#\#}$	1.25±0.33$^{\#\#\#***}$
D-lactate (μg/mL)		
After admission	15.4±3.25	16.0±3.86
1 d postoperatively	42.9±5.26$^{\#\#\#}$	36.5±4.19$^{\#\#\#***}$
7 d postoperatively	36.3±23.4$^{\#\#\#}$	19.6±5.28$^{\#\#\#***}$
DAO (mg/mL)		
After admission	40.3±3.85	41.1±4.19
1 d postoperatively	104±12.9$^{\#\#\#}$	86.7±5.94$^{\#***}$
7 d postoperatively	65.9±6.28$^{\#\#\#}$	53.7±5.98$^{\#\#\#***}$

DAO: diamine oxidase; EN: enteral nutrition; EIN: enteral immunonutrition.
Data are presented as means ± SDs.
$^{\#\#\#}p<0.001$ vs. after admission.
$^{**}p<0.01$ and $^{***}p<0.001$ vs the EN group.

Table 4. Comparison of the enteral feeding tolerance

	EN group (n=50)	EIN group (n=52)
Complete tolerance	18	32
Partial tolerance	25	19
Intolerance	7	1*

EN: enteral nutrition; EIN: enteral immunonutrition.
Data are presented as numbers of patients.
$^{*}p<0.05$ vs the EN group.

Table 5. Comparison of complications

	EN group (n=50)	EIN group (n=52)
Lower extremity deep vein thrombosis	0	1 (1.92)
Incisional infection	1 (2.00)	0
Abdominal infection	1 (2.00)	0
Urinary tract infection	1 (2.00)	1 (1.92)
Anastomotic fistula	1 (2.00)	1 (1.92)
Reflux pneumonia	1 (2.00)	0
Total	5 (10.0)	3 (5.77)

EN: enteral nutrition; EIN: enteral immunonutrition.
Data are presented as n (%).

Klek et al found that EIN shortens the postoperative hospital stay, improves the perioperative nutritional status, and reduces the hospitalization costs.[14] Cui et al found that administration of EIN after surgery can modulate the inflammatory response and enhance the immune function of patients with gastric cancer.[15] In a randomized controlled trial, Suzuki et al observed that postoperative EIN prevented complications and promoted lymphocyte proliferation compared with total parenteral nutrition.[16] In our study, the times to return of the first bowel sound, first postoperative flatus, and first bowel movement were shorter in the EIN group than in the EN group, while the postoperative nutritional status, intestinal flora, intestinal permeability, and enteral feeding tolerance were better in the EIN group than in the EN group; these findings are consistent with those of the above-mentioned study, reaffirming the value of EIN in patients with gastrointestinal diseases. The underlying mechanism may be related to the action of the agents included in EIN, such as the live combined *Bifidobacterium*, *Lactobacillus*, and *Enterococcus* preparation; L-arginine; and glutamine. The live combined *Bifidobacterium*, *Lactobacillus*, and *Enterococcus* preparation contains probiotic bacteria, which can bind to the specific receptors of intestinal epithelial cells, prevent the invasion of other pathogenic bacteria, promote the recovery of the function and structure of the gastrointestinal tract, and enhance the function of the intestinal mucosal barrier. Moreover, they produce antimicrobial agents through metabolism, thus inhibiting or killing pathogenic microorganisms; directly replenish the normal flora of the intestine; aid the reproduction and growth of beneficial bacteria; and inhibit the reproduction of pathogenic bacteria, thus restoring the balance of the intestinal flora.[17,18] L-arginine is an essential mammalian amino acid and a precursor of nitric oxide that can be catalyzed by nitric oxide synthase into nitric oxide; it plays a role in stimulating immune cells, promoting wound healing, improving microvascular perfusion, and repairing tissues. Because trauma, surgery, and other stress-related factors cause negative nitrogen balance, the synthesis of amino acids cannot meet the demand of the body. In this situation, glutamine is consumed in great

quantity, which results in increased intestinal permeability, damaged intestinal mucosal epithelial cells, and impaired intestinal mucosal barrier. Supplementation with exogenous glutamine can maintain the intestinal mucosal structure, weight, and protein content; prevent intestinal mucosal atrophy; improve the intestinal immune function and cell activity; and avoid the translocation of intestinal bacteria/endotoxins.[19] Achamrah et al highlighted the beneficial effects of glutamine on gastrointestinal diseases, including maintenance of the integrity of the intestinal barrier and reduction of the intestinal permeability.[20] Kim and Kim found that glutamine regulates tight junction proteins, promotes enterocyte proliferation, inhibits pro-inflammatory signaling pathways, and protects cells from stress-induced apoptosis and that supplementation with exogenous glutamine attenuates muscle proteolysis, promotes protein synthesis, and improves nitrogen balance.[21]

In this study, the concentrations of IgA, IgG, IgM, complement C3, and complement C4 were higher in the EIN group than in the EN group at 1 and 7 days postoperatively, and no significant differences in the perioperative complications were observed between the two groups; these results indicate that EIN can enhance the postoperative humoral immune function in patients with digestive system diseases, with fewer complications. The underlying mechanism may be related to the following: (1) Omega-3 is integrated into the lipid bilayer of the endothelial cell membrane and T lymphocytes, which can affect the spatial composition of cell membrane receptors, improve the cell membrane composition, change the signal transduction processes, and regulate the expression of interleukins and fatty acids in the process of inflammation, thereby playing a role in regulating the immune function by inhibiting the synthesis of pro-inflammatory factors, such as interleukin-1, interleukin-6, tumor necrosis factor, and prostaglandin E2.[1,22] Plank et al found that preoperative administration of EN preparations containing omega-3 components for 7 consecutive days can maintain the immunity level, reduce the postoperative inflammatory response, and prevent the occurrence of postoperative infections in patients with gastric cancer.[23] (2) Probiotics can interact with dendritic cells and affect the differentiation of T cells into regulatory T cells or Th1 and Th2 cells, inhibit the production of pro-inflammatory factors, such as tumor necrosis factor-α, by monocytes, and play a role in regulating immune responses. Moreover, probiotics can regulate immune responses through dendritic cells and macrophages, regulate the secretion of cytokines by immune cells, stimulate the host immune response, and induce the immune function of the body.[24] (3) Glutamine helps promote the differentiation, mitosis, and proliferation of macrophages and lymphocytes, thus enhancing immune cell replication and protecting the function of immune cells.[25]

In conclusion, EIN can promote gastrointestinal function recovery, improve the nutritional status, enhance the humoral immune function, regulate intestinal flora balance, improve intestinal permeability, and prevent enteral feeding intolerance in patients with digestive system diseases, with few complications after surgery. Owing to the small sample size, short follow-up period, and single source of cases in our study, future studies with a larger sample size are warranted to further explore the possibility of improving the nutritional status and immune function of patients with gastrointestinal diseases.

AUTHOR DISCLOSURES

The authors declare that they have no conflicts of interest.

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Original Article

Effects of fructose from apple and honey on serum uric acid in young Chinese: Randomized crossover trials

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Background and Objectives: Overconsumption of drinks containing fructose increases the risk for hyperuricemia and gout. Comparative analysis evaluating the indicators of serum uric acid (SUA) load caused by natural food-derived fructose and pure fructose in sweeteners is lacking. We aimed to uncover the effect of fructose from apple and honey and pure fructose powder on the SUA concentration of healthy young Chinese individuals. **Methods and Study Design:** Two randomized crossover trials were performed. The participants were randomly assigned to consume apple or honey (test food) or pure fructose powder (reference food); one week later, the groups' dietary intervention was switched. Blood samples were collected at 0, 30, 60, and 120 min after meal to measure the SUA and blood glucose concentrations. **Results:** At 30 and 60 min, the SUA concentration in participants consuming apple or honey was lower than in those consuming fructose powder. At 120 min, the SUA concentration of participants consuming apple returned to baseline. The areas under the curve (AUC) within 2 h (2h-AUCs) of SUA exhibited the trend of fructose >honey >apple. The 2h-AUC ratio between test food and reference food was determined using the uric acid index to assess the efficiency of food-derived fructose in increasing the SUA concentration. The uric acid index of honey was higher than that of apple. Men had higher postprandial SUA concentration than women. **Conclusions:** Food-derived fructose caused a lighter load on uric acid metabolism than pure fructose. Uric acid index can be useful for distinguishing fructose-containing foods.

Key Words: serum uric acid, uric acid index, fructose, apple, honey

INTRODUCTION

Uric acid in blood at low concentration exerts an antioxidative effect according to the human and animal data; however, in excessive amounts, it crystallizes and acts as a pro-oxidant, thereby inducing gout.[1] To date, chronic hyperuricemia is accepted as an independent risk factor for gout,[2] rheumatoid arthritis,[3] nonalcoholic fatty liver disease, diabetes,[4-6] and even some types of cancers.[7,8]

Uric acid is not only derived from the breakdown of purines, but also from fructose metabolism. The hepatic metabolism of fructose leads to the rapid depletion of ATP and increased production of uric acid.[9-12] In the modern food industry, a sweetener rich in fructose is often used in processed foods, including sweetened foods and beverages, since fructose is the sweetest naturally occurring carbohydrate.[13] High fructose corn syrup (HFCS) is a man-made flavored syrup containing a high proportion of fructose and is widely used in manufactured foods. Participants consuming HFCS-sweetened beverage reportedly had higher serum uric acid (SUA) concentration than those consuming sucrose-sweetened beverage. Data from previous prospective cohort studies reported a positive association between the intake of fructose-rich beverages and an increased risk of gout or hyperuricemia in men and women.14,15 A previous meta-analysis showed that the risk of hyperuricemia is positively correlated with the intake of fructose (odds ratio: 1.85; 95% confidence interval: 1.66–2.07).[16] Moreover, a larger body of evidence, including from animal experiments,[17,18] epidemiological studies, and clinical trials, supports that sugary drinks with HFCS also increase the risk of obesity, hypertension, insulin resistance, fatty liver, and dyslipidemia. Therefore, limiting the intake of HFCS is largely recommended.[19-21]

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Notably, fruits and honey are the main sources of natural fructose.[22] The Brisighella Heart Study based on the Mediterranean cohort showed that people who consumed large amounts of fruits had elevated SUA concentration and increased incidence of hypertension and diabetes,[23] low-density lipoprotein oxidation,[24] arterial stiffness,[25] impaired cognitive function,[26] and heart failure.[27] The overconsumption of fructose even from fruits is suggested to be harmful. However, increasing the intake of fruits is recommended by health organizations worldwide as they are low energy-dense foods that are rich in fiber and micronutrients.[28] Therefore, a method for assessing the capacity of fruits to increase the SUA concentration is warranted. Honey is a sweet and viscous food substance. It is often used for cooking and baking, or as a spread on bread in Western countries; it is also used in some commercial beverages such as tea drink in China.

This study aimed to compare the effect of fructose from apple, honey, and pure fructose powder on the SUA concentration of healthy young Chinese individuals and explore a method to evaluate the load on uric acid metabolism caused by natural food-derived fructose.

METHODS

Study design

The study was performed at the School of Medicine, Ningbo University, from September to October 2020. Two randomized crossover trials, apple trial and honey trial, were carried out: apple or honey was used as test food, while a commercial pure fructose powder was used as a reference food. In the apple trial, the participants were randomly assigned to consume apple (test food) or pure fructose powder (reference food) at the first stage, and received an alternative dietary treatment at the second stage after a washout period of one week. In the honey trial, the test food was honey, while the reference food was pure fructose powder; the participants received the dietary intervention following the same principle. If the sample size is 16 persons, an 80% power is required to detect a difference of 1 standard deviation (SD) in plasma uric acid response to treatment at an α level of 0.05, since 20% of the participants is expected to drop out from the study. Therefore, the sample size exceeds 20 persons.[29]

Participants

College students were recruited from Ningbo University. Participants 1) aged 18–26 years, 2) with a fasting blood glucose concentration of <6.1 mmol/L, and 3) with a body mass index of <24 kg/m^2 according to the recommendation of the China Nutrition Society were included in this study. Participants with 1) menstruation and 2) chronic diseases, including diabetes, hypertension, cancers, digestive disorders, gout, and hyperuricemia, which was defined as a fasting SUA of >416.5 μmol/L (men) or 357 μmol/L (women) on two tests were excluded.30 Written informed consent was obtained before the trials were conducted. The experiment was approved by the Ethics Committee of School of Medicine, Ningbo University (Ningbo, China), and registered in the Chinese Clinical Trial Registry at http://www.chictr.org.cn/ (registration number: ChiCTR2000036443).

Trial foods

The same batch of red Fuji apples and Guanshengyuan honey produced by a local manufacturer were used as test foods. Fructose powder with a purity of ≥99% was purchased from Shandong Xiwang Sugar Co. (Shandong, China) and was used as the reference food. At the beginning of trial, honey, fructose powder dissolved in 50 mL warm water, and fresh apple pulp were provided to the participants.

Measurement of fructose in foods using HPLC

HPLC reagents and conditions

The amount of fructose, glucose, and sucrose in foods was measured using high-performance liquid chromatography (HPLC) according to the China National Standard protocol GB 5009.8-2016. A Waters 2695 HPLC apparatus equipped with 2414 differential refractive index detector (Waters Corp, USA) was used. Glucose, fructose, and sucrose standard substances were purchased from Chem Service (West Chester, USA). Zinc acetate and potassium ferrocyanide of analytical grade were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). A 20 μL sample was injected into a Kromasil 100A NH2 column at a temperature of 40 °C (250 × 4.6 mm, 5 μm; Kromasil) and detected using a refractive index detector at a temperature of 35 °C. The elution was performed using acetonitrile and water (75:25, v/v) at a flow rate of 1.0 mL/min.

Sample preparation

Three randomly chosen red Fuji apples were equally divided into three layers (inner, middle, and outer) after removal of peels and seeds. Equal amounts (5 g) of flesh from each layer of the three apples were mixed with the solution (2.5 mL of zinc acetate solution [$Zn(CH_3COO)_2 \cdot 2H_2O$], 2.5 mL of potassium ferricyanide solution {$K_4[Fe(CN)6] \cdot 3H_2O$}, and 45 mL of ultra-pure water) and shaken for 60 min. After centrifugation at 9,500 rpm for 5 min, the mixtures were filtered through a 0.22-μm nylon filter (Millipore). Honey (30 g) from three individual bottles was directly added to sterile tubes.

Calibration curve

A series of dilutions with concentrations of 0.4, 2.0, 5.0, 10.0, and 20.0 mg/mL were prepared and then detected by HPLC to establish the calibration curve of fructose, glucose, and sucrose. The calibration curve was plotted with the peak area on the vertical (Y) axis and the standard concentrations on the horizontal (X) axis using linear regression.

Study protocol

The participants were prohibited from drinking alcohol, consuming oversized animal meat, eating desserts, or staying up late the day before the test. All participants fasted from 10:00 pm the day before the test. On the actual test day, they arrived at the scene at 08:00 am. The height, body weight, and blood pressure of the participants were recorded. Then, the dietary treatment was administered orally. During the dietary intervention, all participants were asked to consume their food within 10 min. Three milliliters of venous blood samples were collected

at 0, 30, 60, and 120 min in order to analyze the uric acid concentration. Blood pressure was also measured at 120 min.

Statistical analysis

All data were analyzed using SPSS 24.0 software (Chicago, IL, USA). The parameters obtained at 0 min of dietary intervention, uric acid index, and 2h-AUC of SUA are expressed as mean ± Standard deviation (SD), while other data were presented as mean ± standard error of means (SEM). The differences in the SUA and blood glucose concentrations between 0 min and 30 min or between 30 and 120 min after a specific treatment were analyzed using repeated measures of the general linear model, while the values obtained at 0 min were adjusted as covariates to avoid the interference of differences between individuals; the sequence of dietary intervention and participants as random effects were adjusted. The 2h-AUC of serum uric acid and blood glucose after administering the dietary treatment were calculated using the trapezoidal rule (GraphPad Prism 8.0.1, San Diego, CA, USA). The normality of variables such as 2h-AUC of uric acid, 2h-AUC of blood glucose, and the changes in blood pressure (both diastolic and systolic) from 0 min to 120 min was assessed using the Shapiro-Wilk test. Normalized and non-normalized data were analyzed using one-way analysis of variance and non-parametric test, respectively. Independent-samples t-test was used to determine the uric acid index. A two-tailed p value of <0.05 was considered statistically significant.

RESULTS

Quantification of fructose with HPLC

The result of HPLC showed that 1 g of Fuji apple provides 0.110 g of fructose (0.096 g of free fructose, 0.041 g of glucose, and 0.0311 g of sucrose comprising 0.014 g of glucose and 0.014 g of fructose), while 1 g of honey has 0.489 g of free fructose and 0.442 g of glucose. Considering the feasibility of intake, the dose of fructose consumed was 25 g, which is equivalent to 222 g of flesh from a medium-sized apple or 51 g of honey.

Anthropometric characteristics of the participants

A flow chart of the study is presented in Figure 1. Twenty-nine and thirty-five college students aged 22–26 years were enrolled in the apple trial (n=29) and honey trial

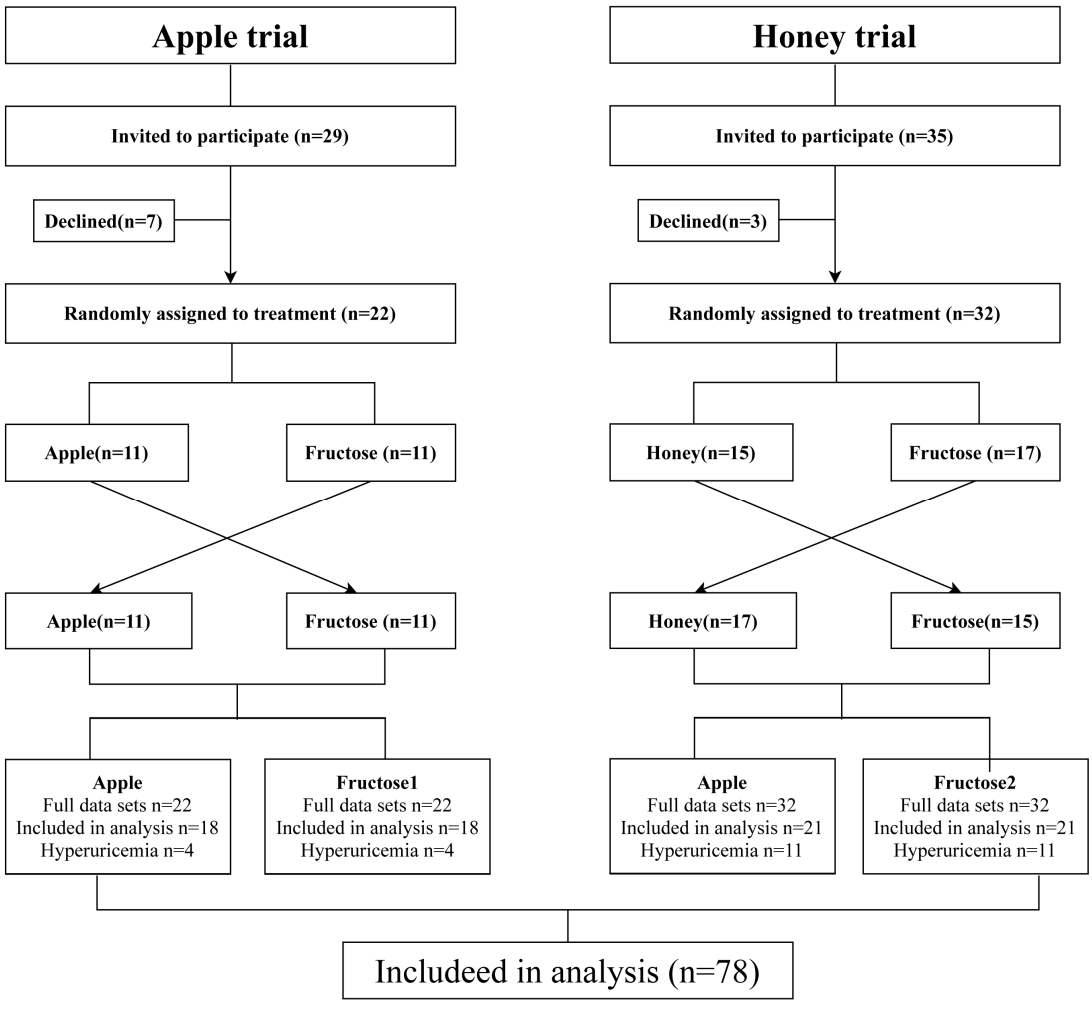

Figure 1. The Consolidated Standards of Reporting Trials diagram of the participant recruitment process. Sixty-four college students (apple trial, n=29; honey trial, n=35) aged 22–26 years were enrolled. Prior to the administration of the dietary intervention, seven (men) and three (two men and one woman) volunteers dropped out of apple trial and honey trial, respectively, due to unrelated personal reasons. Four participants (three men and one woman) in the apple trial and 11 participants (eight men and three women) in the honey trial were excluded from the analysis owing to the occurrence hyperuricemia. Overall, 78 full datasets were included in the study.

(n=35), respectively. Prior to the administration of the dietary intervention, seven (men) and three (two men and one woman) volunteers dropped out of the apple trial and honey trial, respectively, due to unrelated personal reasons. Four participants (three men and one woman) in the apple trial and 11 participants (eight men and three women) in the honey trial were excluded owing to the occurrence of hyperuricemia. The anthropometric characteristics of participants at baseline are summarized in Table 1; no difference was found in all measured parameters at baseline among the participants.

SUA concentration of participants

The changes in SUA concentration of all participants consuming apple, honey, and fructose powder are depicted in Figures 2A and Table 2. As shown in Figures 2A, the SUA concentration of participants after consumption of apple rapidly peaked during the first 30 min, but then gradually decreased to the lowest at 120 min; meanwhile, the SUA concentration of participants consuming honey continuously increased. Of note, the curves of SUA over time after fructose powder and apple consumption had the same shape but different amplitude. After comparing SUA concentration at different time points, results showed that the fructose powder intake induced a significant increase in SUA concentration at 30 and 60 min compared with that at 0 min (fructose (apple trial): $p_{30min}<0.001$, $p_{60min}<0.001$; fructose (honey trial): $p_{30min}<0.001$, $p_{60min}<0.001$). However, no significant difference was found between the SUA concentrations at 30 and 60 min after consuming apple and honey and the SUA concentration at 0 min (apple: $p_{30min}=0.075$, $p60min=0.148$; honey: $p_{30min}=0.057$, $p_{60min}=0.052$). In our study, the difference between SUA test food and SUA reference food (i.e., SUA test food – SUA reference food) of each participant at the same time points after meal was calculated to compare the body's response to fructose derived from different sources. As shown in Table 2, all the values of the SUA test food and SUA reference food at 30 min and 60 min for the apple trial and honey trial were negative. Results of the statistical analysis showed that the SUA concentration of women at 30 min and 60 min after consuming apple were significantly lower than those of fructose ($p_{30min}=0.01$, $p_{60min}=0.02$).

Moreover, men had a stronger response to fructose intake than women, with a higher concentration of SUA at the same time point compared with women, as illustrated in Figure 2B and Figure 2C. The difference between SUA men and SUA women (SUA men–SUA women) was used to quantitatively evaluate the effect of gender on uric acid metabolism. As shown in Table 3, the SUA concentrations of men at 60 min and 120 min were higher than that of women, and a significant difference in SUA was observed between women and men at 120 min after fructose intake in the honey trial ($p=0.04$).

Then, the 2h-AUC of SUA after dietary fructose loading was measured. As shown in Table 4 and Figure 2D and 2E, male and female participants consuming apple and honey had lower 2h-AUC of SUA than those consuming fructose powder. The 2h-AUC of SUA of men consuming apple and honey was significantly higher than that of women (p value for apple intake: was 0.005, p value for honey intake: 0.015). In terms of SUA concentration, men exhibited a stronger response to fructose-containing foods than women. The gender-related difference in fructose treatment almost disappeared when the participants consumed fructose powder.

In this study, a new metabolic parameter was introduced, uric acid index, which was defined as the ratio of $2h\text{-}AUC_{test\ food}$ to $2h\text{-}AUC_{reference\ food}$, to assess the uric acid metabolic load caused by specific food-derived fructose. Both test food and reference food provided 25 g of fructose. Therefore, the uric acid index was calculated and compared using the independent samples t-test. The uric acid index of honey was higher than that of apple, although no significant difference in the uric acid index was observed between apple and honey ($p=0.153$) as depicted in Figure 3F and Table 5. For either men or women, no significant difference was also found in the uric acid index of apples and honey ($p_{women}=0.294$, $p_{men}=0.419$).

Blood glucose measurement

As depicted in Figure 3A, the blood glucose concentration increased significantly at 30 min after the intake of honey and apple, while it only slightly increased after consumption of fructose powder. The highest blood glucose concentration (5.55 μmol /L) was observed in the apple-treated group at 30 min, and no difference was observed in the blood glucose concentration between the honey group and apple group ($p=0.151$). Relatively, the blood glucose concentration of participants who consumed fructose powder was the lowest. At 60 min, the blood glucose concentration of participants who consumed honey was the highest ($p<0.001$), while those of participants who consumed apple and fructose powder had similar blood glucose concentration. The 2h-AUC of blood glucose was also calculated. The 2h-AUC of honey was significantly higher than that of apple and fructose

Table 1. Anthropometric characteristics of participants†

Parameters	Apple trial		Honey trial	
	Apple (n=18)	Fructose (n=18)	Honey (n=21)	Fructose (n=21)
Age (y)	22.5±2.2	22.5±2.2	22.8±1.7	22.8±1.7
women, n (%)	12 (66.7)	12 (66.7)	12 (57.1)	12 (57.1)
men, n (%)	6 (33.3)	6 (33.3)	9 (42.9)	9 (42.9)
BMI ($kg/m^{2)}$	20.7±1.72	20.7±1.72	20.2±2.05	20.2±2.05
Systolic blood pressure (mm Hg)	116±11.6	115±10.18	110±8.54	111±11.3
Diastolic blood pressure (mm Hg)	78.4±9.00	80.2±8.33	70.8±7.96	70.1±7.66
Blood glucose (mmol/L)	4.09±0.38	4.17±0.24	4.17±0.61	3.92±0.57

DBP: diastolic blood pressure; SBP: systolic blood pressure.
†Data were obtained at 0 min of dietary intervention and presented as mean±SD.

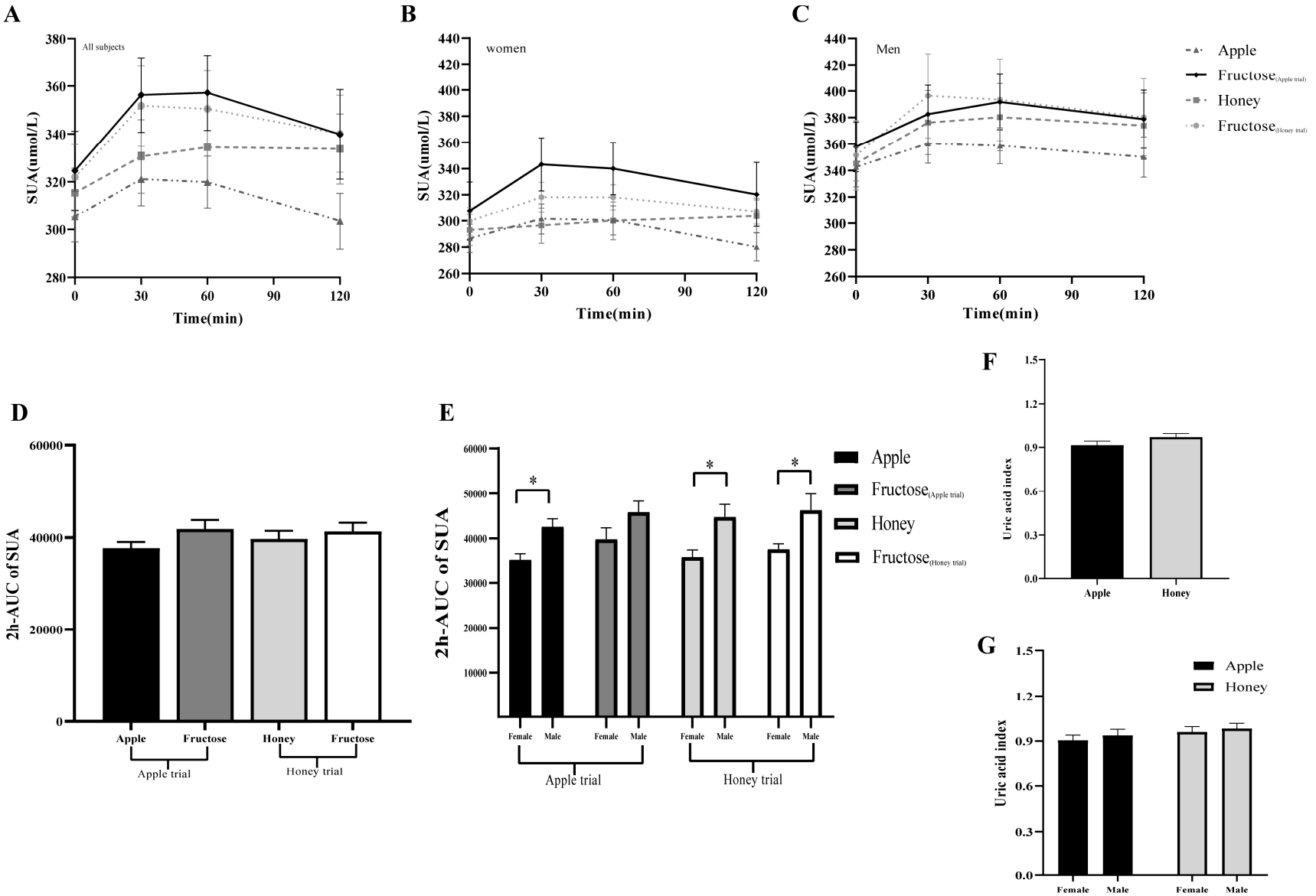

Figure 2. Serum uric acid (SUA) concentration after consuming 25 g of fructose derived from different sources. A: SUA concentration of all participants at 0 min, 30 min, 60 min, and 120 min. B: SUA of women at 0 min, 30 min, 60 min, and 120 min. C: SUA of men at 0 min, 30 min, 60 min, and 120 min. D: Area under the curve (AUC) for SUA in all participants. E: AUC for SUA in women and men. F: Uric acid index in all participants. G: Uric acid index in women and men. $^{*}p<0.05$.

Table 2. Serum uric acid concentration at different time points after food intervention[†]

		SUA at 0min, μmol/L[‡]			SUA test food –SUA reference food, μmol/L[‡]					
	Gender	SUA Test Food	SUA Reference food	n	30 min	$p^{§}$	60 min	$p^{§}$	120 min	$p^{§}$
Apple trail	women	287±37.0	308±75.8	24	-22.5±8.41*	0.01	-20.6±7.74*	0.02	-18.6±10.4	0.09
	men	343±36.8	358±45.8	12	-6.91±11.3	0.56	-18.2±9.03	0.07	-12.4±9.00	0.20
	All subjects	305±13.5	325±13.5	29	-35.2±22.5	0.54	-37.3±22.3	0.63	-36.3±22.7	0.51
Honey trail	women	293±40.8	300±36.8	24	-13.5±7.45	0.08	-12.0±7.97	0.15	2.80±6.48	0.67
	men	345±39.2	351±78.6	18	-12.6±16.9	0.47	-5.80±20.3	0.78	1.05±17.4	0.95
	All subjects	315±12.5	323±12.8	42	-22.3±21.1	0.88	-18.7±20.9	0.89	-6.39±21.3	1.00

[†]Participants with complete data (0 min, 30 min, 60 min, and 120 min) and normal uric acid concentration (men: <416.4 μmol/L; women: <356.9 μmol/L) were enrolled.
[‡] Data at 0 min were presented as mean ± SD; results at 30 min, 60 min, and 120 min were presented as mean ± SEM.
[§]General linear model: repeated measures analysis was used to compare the effects of dietary treatments. The treatments were considered as fixed effects, while the participants were considered as random effects; values at 0 min were included in the model as covariates.
*p<0.05.

Table 3. Comparison of SUA concentration at different time points after meal by gender[†]

	Foods	n	SUA men –SUA women at 30 min[‡]	$p^{§}$	SUA men –SUA women at 60 min[‡]	$p^{§}$	SUA men –SUA women at 120 min[‡]	$p^{§}$
Apple trail	Apple	18	2.69±7.92	0.74	5.09±8.38	0.55	18.7±9.95	0.08
	Fructose	18	-5.36±13.4	0.70	7.19±11.3	0.54	5.54±14.6	0.71
Honey trail	Honey	21	13.0±16.1	0.43	12.5±19.0	0.52	5.30±16.5	0.75
	Fructose	21	22.0±11.8	0.08	21.0±12.8	0.12	21.4±10.6*	0.04

[†]Participants with complete data (0 min, 30 min, 60 min, and 120 min) and normal uric acid concentration (men: <416.4 μmol/L; women: <356.9 μmol/L) were enrolled.
[‡]Results at 30 min, 60 min, and 120 min were presented as mean ± SEM.
[§]General linear model: repeated measures analysis was used to compare the SUA concentrations at different time points of food intake by gender. Gender was considered as fixed effects, while the participants were considered as random effects; values at 0 min were included in the model as covariates.
*p<0.05.

Table 4. 2h-AUC of SUA of apple, honey by gender[†]

	FOODS	2h-AUC men[‡]	2h-AUC women[‡]	n	$p^{§}$
Apple trail	Apple	42595±4222**	35270±4562	18	0.005
	Fructose	45828±6044	39824±8748	18	0.153
Honey trail	Honey	44788±8285*	35899±5350	21	0.015
	Fructose	46273±10731*	37609±4065	21	0.047

[†]AUC of uric acid was calculated by GraphPad Prism software using the trapezoidal method.
[‡]All data were presented as mean±SD.
[§]The normality of data distribution was assessed using Shapiro-Wilk test. The data with normal and non-normal distribution were analyzed using one-way analysis of variance and non-parametric test, respectively.
*p<0.05, **p<0.01 when compared to 2h-AUC women.

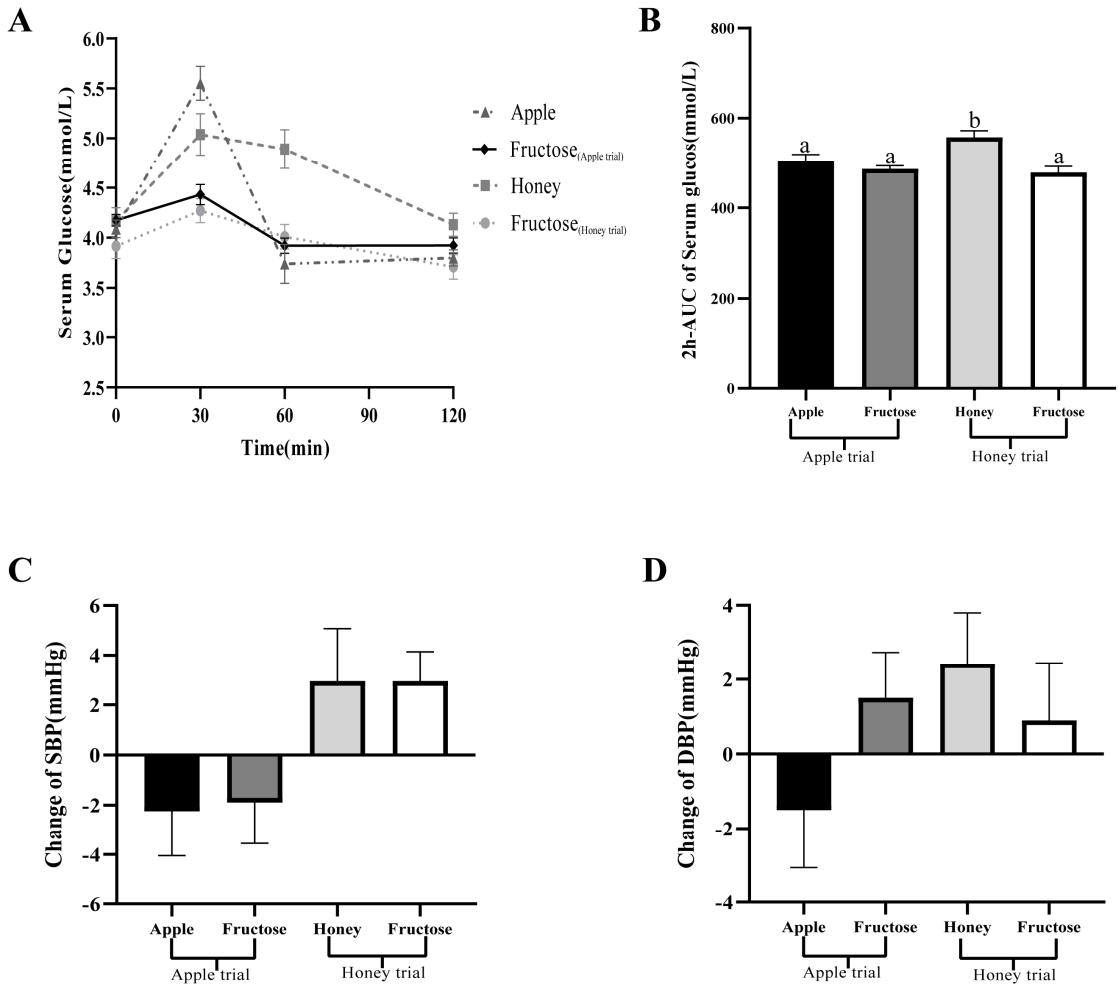

Figure 3. Effect of apple, honey, and fructose consumption on blood glucose and blood pressure. A: Blood glucose concentration at 0 min, 30 min, 60 min, and 120 min. B: Area under the curve of blood glucose. C and D: Changes in systolic blood pressure and diastolic blood pressure between 0 min and 120 min. Values without a common superscript are significantly different; $p<0.05$.

Table 5. The uric acid index of apple and honey

Gender	Testing Food	n	Uric acid index†	p‡
Woman	Apple	12	0.90±0.12	0.294
	honey	12	0.96±0.13	
Men	Apple	6	0.94±0.10	0.419
	honey	9	0.98±0.10	
All subjects	Apple	18	0.91±0.12	0.153
	honey	21	0.97±0.12	

All data were presented as mean±SD.
†Uric acid index was newly defined as the ratio of 2h-AUC of the test food (contain 25 g fructose) to that of the reference food (pure fructose powder, 25g).
‡The normality of data distribution was assessed using Shapiro-Wilk test. The data with normal and non-normal distribution were analyzed using independent-samples T-test and non-parametric test, respectively.

($p<0.01$), as shown in Figure 3B.

Blood pressure evaluation

The effect of food containing fructose on blood pressure is shown in Figure 3C and 3D. No significant difference was observed in the change in diastolic or systolic blood pressure after the ingestion of foods, although the consumption of apples tended to reduce the systolic and diastolic blood pressure.

DISCUSSION

Earlier studies have shown that purine-rich foods such as meat, seafood, and alcohol are important dietary factors contributing to the development of hyperuricemia.[31] In recent decades, the consumption of fructose-rich drinks has gained increased attention in relation to the overproduction of uric acid as a byproduct of fructose metabolism.[32] Our found that acute intake of fructose-containing foods such as apples, honey, and fructose powder induced a significant increase in SUA concentration in all participants, regardless of the source of fructose. This finding is consistent with that of a previous study, which indicated that the consumption of apples and apple juice containing fructose increased the SUA concentration in an American

population;[29] in that study, the amount of fructose consumed (26.5 g) was similar to that consumed in our study (25 g). Fructose is a monosaccharide, with a metabolic pathway in the human body that differs greatly from that of glucose, although it is an isomer of glucose. During glucose metabolism, hexokinase catalyzes glucose into glucose-6-phosphate, which then modulates the activity of hexokinase via negative feedback, thereby preventing the overproduction of glucose metabolites. However, fructose metabolism lacks this negative feedback mechanism. When a large amount of fructose was consumed, its rapid metabolism led to the breakdown of adenosine triphosphate (ATP) and phosphate to generate adenosine diphosphate and adenosine monophosphate (AMP). The rapid decrease in phosphate stimulates the production of AMP deaminase, which in turn catalyzes the transformation of AMP to hypoxanthine nucleotide and ultimately to uric acid.[33] The intracellular concentration of uric acid increased and was released into the circulation; as a result, the SUA concentration peaked at 15 min to 1 h after ingestion.[34] Animal and human studies revealed that increase in the SUA concentrations mediated the adverse effects of fructose ingestion in our body's metabolism.[35,36] A previous clinical trial showed that consumption of fructose-containing beverages for 10 weeks significantly elevated the postprandial SUA concentration compared with isocaloric intake of glucose.[37] The ratio of fructose to glucose (F:G) in beverages has a great influence on SUA concentration; that is, consumption of a large amount of fructose increases the SUA concentration. A previous study conducted in male participants showed that an F80:G20 drink induced a higher serum concentration of uric acid than a G50:F50 drink.[38] Therefore, it is necessary to limit the ordinary intake of fructose in soft drinks to maintain health. Some scholars even warned that gouty participants should avoid consuming fruits.[39]

Our data demonstrated that the ingestion of pure fructose rapidly increased the uric acid concentration and resulted in the highest uric acid 2h-AUC; moreover, the 2h-AUC of SUA after apple intake was the lowest among the three dietary treatments. Fruits are enriched with bioactive compounds such as vitamin C and polyphenol, and robust epidemiological evidence showed the beneficial effects of fruit consumption on health.[40] The slow response of the human body to apple intake may be related to its plentiful bioactive substances, including fiber, vitamin C, and antioxidants such as polyphenols.[34,38,41] Polyphenols were found to interfere with intestinal sugar transporters, and result in glucose and fructose absorption.[42] Fibers derived from natural fruits can slow down the digestion of fructose in the small intestine,[43] while vitamin C enhances the urinary urate excretion.[44] We initially proposed the definition of uric acid index and pointed out that it is more optimal for the general population and gouty patients to choose foods based on the uric acid index rather than completely eradicating fruits from their diet. In our study, honey also induced a slow increase in the SUA concentration like apple; in contrast to pure fructose, the slow increase in the SUA concentration may be associated with the presence of phenols in honey, which may impede the intestinal sugar transporters. Our data showed that the uric acid index of honey is higher than that of apple, suggesting that apples may be safer for gouty participants to consume than honey.

Moreover, men exhibited a more rapid increase in the SUA concentration after fructose consumption than women, and the SUA concentration of men did not return to baseline at 120 min. By contrast, the SUA concentration of women returned to baseline by 120 min. The incidence of hyperuricemia and gout in men was four times higher than that in women.[45] Postmenopausal women who did not undergo hormone replacement therapy had high concentrations of SUA;[46] meanwhile, exogenous estrogen intervention decreased the SUA concentrations in postmenopausal women. An epidemiological study in Taiwan conducted in 5,896 participants (2,960 women and 2,936 men) reported that the SUA concentration in women increased with age.[47] The abovementioned studies suggested that estrogen has a positive effect on uric acid metabolism. Moreover, women had stronger capacity to handle the load of dietary fructose. However, there are some limitations in this study. First, this study included healthy young adults, and the response of individuals with gout or compromised kidney function remains unclear. Second, this was a short-term trial; hence, long-term intervention trials are warranted to assess the health effect of fructose from different sources. Finally, due to financial and logistical constraints, two trials were carried out in two groups of participants; however, the serum uric acid responses of these healthy young adults to the same amount of fructose were similar, which strengthens the generalizability of our results.

Conclusion

This study was the first to demonstrate that the consumption of the same amount of fructose derived from different food sources triggered the elevation of SUA concentration to different extents as shown in Figure 4, exhibiting the trend of fructose powder > honey > apple. A gender difference was found in the metabolism of fructose; the SUA concentration of men exhibited a more rapid increase after fructose consumption compared with that of women. Hence, it is meaningful to establish fructose intake limits by gender. Uric acid index may be a useful tool to guide hyperuricemia or gout patients in choosing appropriate foods that they can consume.

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AUTHOR DISCLOSURES

The authors declare no conflicts of interest.

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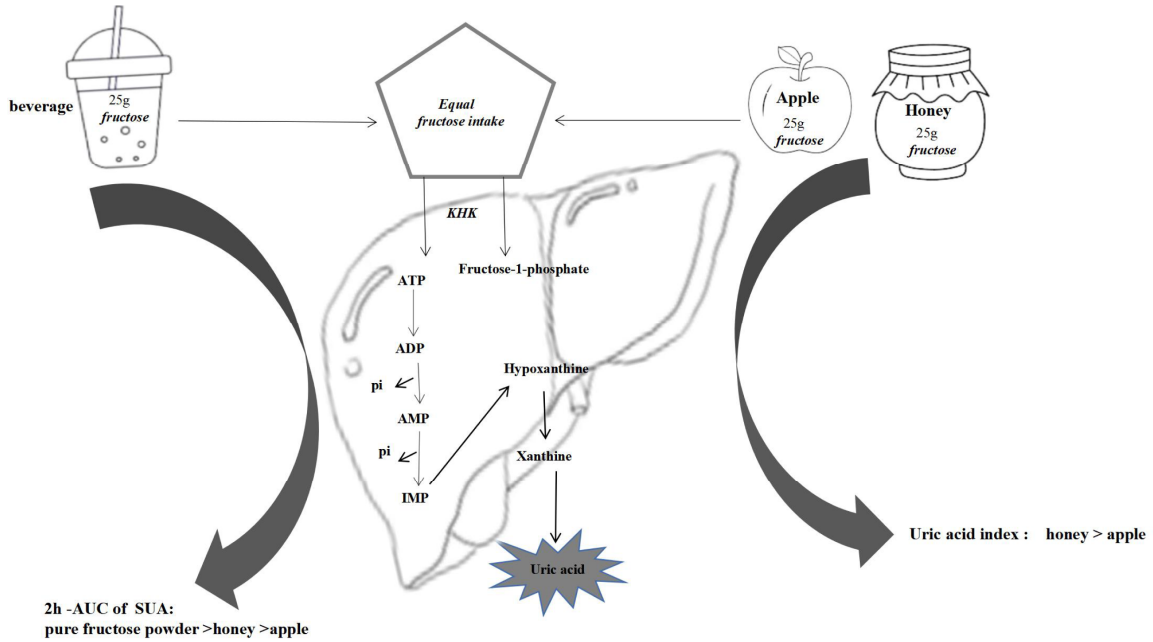

Figure 4. Conceptual diagram of various sources of fructose caused an elevation of serum uric acid concentration to different extent. Fructose readily absorbed can be rapidly metabolized by fructokinase in liver to produce fructose-1-phosphate, leading to a depletion in intracellular ATP and generation of IMP. Then IMP is further degraded to hypoxanthine and xanthine, that is used by xanthine oxidase to generate end-product uric acid. Abbreviations: KHK, ketohexokinase; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; SUA, serum uric acid; AUC, the areas under the curve.

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Original Article

Branched chain and other amino acid intakes are inversely associated with sarcopenia among community elders in Qingdao, China

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Background and Objectives: The present study aimed to investigate the hypothesis that dietary amino acid intakes are associated with the risk of sarcopenia through a community-based observational study. **Methods and Study Design:** A total of 1,140 participants (72.7±6.3 y) were recruited from an annual health check-up program in Qingdao, China. Skeletal muscle mass, muscle mass functions and biochemical parameters were measured by standard methods. Dietary intake was assessed by 3-day, 24-hour food records. The odds ratios (ORs) and 95% confidence intervals (CIs) of sarcopenic risk across quartiles of amino acid intakes were calculated using a multivariable-adjusted logistic regression model. Generalized linear models were used to assess the associations between dietary amino acid intakes and muscle mass functions. **Results:** The prevalence of sarcopenia was 4.1%. Compared with the lowest category intake, the highest category of branched chain amino acids (BCAAs) (OR=0.11; 95% CI: 0.01, 0.90; *p* for trend=0.119), isoleucine (OR=0.11; 95% CI: 0.01, 0.89; *p* for trend=0.122) and tryptophan (OR=0.10; 95% CI: 0.01, 0.87; *p* for trend=0.176) was negatively correlated with sarcopenic risk with adjustment for potential confounding factors. Generalized linear model analysis showed that gait speed was positively correlated with dietary intakes of lysine, threonine, leucine, valine, tryptophan, BCAAs and aromatic amino acids ($p<0.05$). **Conclusions:** Higher intakes of BCAAs were associated with a lower risk of sarcopenia, which might beneficially protect against sarcopenia and improve physical function of the elderly.

Key Words: sarcopenia, muscle mass functions, branched chain amino acid, protein, amino acids

INTRODUCTION

Sarcopenia is an age-related muscle mass loss accompanied by a reduction in muscle strength and/or physical performance.[1] The pathogenesis of sarcopenia is complex, involving changes in body composition and hormones related to aging, inflammatory response, insulin resistance and mitochondrial dysfunction.[2] With the increase in muscle loss, the risks of falls, physical disability, depression, cardiovascular disease, hospitalization and even death have been substantially increased in the elderly. When sarcopenia is associated with malnutrition, chronic inflammation, liver cirrhosis, renal function disease or vitamin D deficiency, the quality of life is inevitable to be further compromised.[3-9] Thus, improving muscle mass and functions are a meaningful public health issue.[10-12]

Cross-sectional surveys have found the prevalence of sarcopenia to be between 0.4% and 13.9%. Age, gender, body mass index (BMI), dietary energy and protein, and physical activity were identified as independent risk factors of sarcopenia.[13-17] However, the results have been inconsistent. The findings from epidemiological studies showed that malnutrition and hypoproteinemia exerted vital roles in patients with sarcopenia,[5] and insufficient dietary protein intake was positively correlated with the risk of sarcopenia, contributing to the decline of body muscle mass.[18] Furthermore, supplemental essential amino acids could enhance muscle protein synthesis,[19] and improve muscle quality, physical function and quality of life. Specifically, branched chain amino acids (BCAAs), including leucine, isoleucine and valine, are considered to improve skeletal muscle atrophy induced by angiotensin II.[20,21]

In China and Japan, 30% of the residents living in the community had the risk of malnutrition or malnutrition,

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and the risk of malnutrition was higher among the elderly over 70 years.[22] To date, no study has been conducted to investigate the associations of dietary protein and amino acid intakes with risk of sarcopenia. Meanwhile, the relationships remained inconclusive between amino acid intakes and muscle mass functions. In the present study, we hypothesized that different amino acids have differential functions associated with the risk of sarcopenia in the elderly. Therefore, we conducted the present study to illuminate these relationships according to residents over 65 years old in the Qingdao community.

METHODS

Study design and participants

Community-dwelling of Qingdao residents (Fushan and Ningxia Community) aged 65 and over were screened and enrolled between Mar. and Nov. 2020. The recruiting process is shown in Figure 1. This study was conducted according to the Declaration of Helsinki and approved by the Ethics Committee of Affiliated Hospital of Qingdao University (QYFYWZLL25549), and written informed consent was obtained from all subjects.

To guarantee the accuracy of the results, the participants were excluded: (1) participants with stroke, parkinsonism, malignant tumor, and chronic kidney disease; (2) participants with factors that affected the gait speed, grip strength and bioelectrical impedance analysis (BIA) measurement, such as implanted with metal, pacemaker, severe edema or physical disability; and (3) participants with severe cognitive impairment or those with poor compliance.

Sarcopenia determination

According to the diagnostic criteria defined by the 2019 Asian Working Group for Sarcopenia (AWGS),[1] the participants with sarcopenia were screened in Qingdao, China. Bioelectrical impedance analysis (BIA; InBoyS10, Korea) was applied to measure muscle mass. The appendicular skeletal muscle mass (ASM) was calculated as the sum of lean muscle mass in the arms and legs. Appendicular skeletal muscle mass index (ASMI) was calculated as the ratio of ASM to the square of height (ASM/height^2). According to the 2019 AWGS consensus, the standard of low muscle mass for males was ASMI <7 kg/m^2, and for females was ASMI <5.7 kg/m^2. Grip strength was measured using a grip strength meter to indicate muscle strength. The maximum grip strength of the dominant hand was measured three times with an interval of one minute, and the maximum value was recorded. The muscle strength was less than 26 kg for males and 18 kg for females defined as a reduction in grip strength. The 6-meter (meter per second, m/s) walking test was conducted to measure the gait speed with normal walking speed. Gait speed <1 m/s was defined as a decrease in physical function. Based on the diagnostic criteria of the 2019 AWGS, sarcopenia was defined as a decrease in the ASMI accompanied by a decrease in muscle strength and/or physical function.[1]

Dietary assessment

The dietary intakes of protein and amino acids were calculated based on 3-day (2 working days and 1 weekend), 24-hour food records.[15] Prior to the survey, participants were instructed how to correctly record dietary intakes and estimate the amount of liquid and solid foods. The nutrition system of traditional Chinese medicine combined with western medicine (NCCW version 12.0) was applied to yield daily intakes of total energy intake (kcal), carbohydrate intake (g), fat intake (g), protein intake (g), and each amino acid intake (mg). Investigators and dietary recorders had received professional guidance and training before conducting investigation and dietary analysis.

Other variables

Demographic parameters and lifestyles were collected based on a face-to-face questionnaire. According to the current smoking status, the participants were divided into

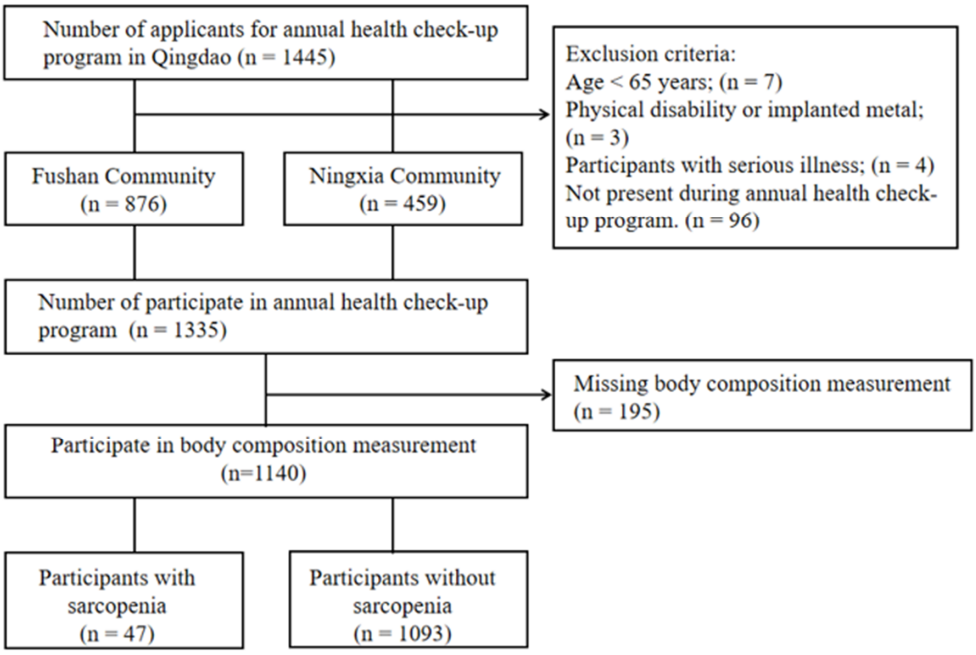

Figure 1. The flow chat of the included participants.

smoking, quitting and never smoking. The participants were defined as alcohol drinking if the females drank 70 grams per week and males drank 140 grams per week. The physical activity scale for the elderly (PASE) was used to evaluate the physical activity of the participants.[23] According to the scores of the PASE, the participants were divided into the low-impact exercise group (<33rd percentile), the moderate-impact exercise group (33rd-66th percentile) and the high-impact exercise group (>66th percentile).

After 10 h fasting, the waist circumference, hip circumference, height and weight of the participants were measured by a trained staff. Accordingly, BMI was calculated as weight divided by height squared (kg/m^2). Meanwhile, blood samples (5 mL) were obtained into vacuum tubes for laboratory analysis. Serum was collected after centrifugation at 3500 rpm for 10 min at 4 °C. Then, serum fasting glucose, total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured by an automatic biochemical analyser (TBA-40FR, Toshiba, Japan) using an enzyme-based colorimetric test.

Statistical analysis

Statistical analysis was performed with STATA version 15.0. After the normality test of the continuous variable data, the quantitative data with normal distribution and non-normal distribution were expressed as mean ± standard deviation (SD) and median (Q25, Q75), respectively. Categorical variables were presented as frequencies (percentages). The continuous parameters with normal distribution between groups were compared with the Student's t-test. Besides, the categorical and non-normally distributed variables were analysed with Pearson's chi-square and Wilcoxon rank sum test, respectively.

Multivariable-adjusted logistical regression models were adopted to estimate odds ratios (ORs) with 95% confidence intervals (CIs) of sarcopenic risk across quartiles of protein and amino acid intakes, with the lowest category as the reference.[24] Tests for trends were conducted by assigning the median value for each category and modelling this variable as a continuous variable. The generalized linear model (GLM) was used to analyse the associations of daily protein and amino acid intakes with muscle mass functions. The multivariable-adjusted models of logistic regression and generalized linear analyses were adjusted for age, gender, height, weight, BMI, waist circumference, hip circumference, waist hip ratio, blood pressure, marital status, education level, smoking, alcohol drinking, physical activity, fasting blood glucose, TC, TG, HDL-C, LDL-C, and for daily energy, carbohydrate, and fat intakes. The two-tailed p values <0.05 were considered as statistically significant.

RESULTS

Participants Characteristics

A total of 1335 participants aged 73.0±6.8 years were recruited in this study. Of these, 1,140 participants completed the BIA measurement, and were included for data analysis. According to the 2019 AWGS consensus, 47 participants were regarded as sarcopenia, and the prevalence of sarcopenia was 4.1%, including 13 males and 34 females.

The characteristics between healthy and sarcopenic participants

The average age of participants with sarcopenia was significantly higher than that of healthy participants (p<0.001). The prevalence of sarcopenia was significantly higher in females than males (p=0.022). Compared with healthy participants, patients with sarcopenia had a higher widowhood rate (p<0.001) and a lower alcohol drinking rate (p=0.007). Meanwhile, the height, weight, BMI, waist circumference and hip circumference of sarcopenic patients were significantly lower than those of healthy participants (p<0.001). In addition, the levels of serum TC (p=0.050) and HDL-C (p=0.036) in sarcopenic patients were significantly higher than healthy participants, while the levels of serum TG (p=0.023) were significantly lower than those healthy participants. Besides, the healthy participants showed significantly higher intakes of energy (p=0.026), protein (p=0.013) and carbohydrate (p=0.031) compared with sarcopenic patients (Table 1).

Dietary factors associated with sarcopenic risk

In univariate logistic regression analyses, dietary intake of protein in the third category was associated with a lower risk of sarcopenia (OR=0.19; 95% CI: 0.05, 0.67; p for trend=0.340), and the association remained significant with adjustment for potential confounding factors (OR=0.02; 95% CI: 0.01, 0.53; p for trend=0.056) (Figure 2A). Regarding amino acids, dietary intakes of branched chain amino acids (OR=0.11; 95% CI: 0.01, 0.90; p for trend=0.119) (Figure 2B), isoleucine (OR=0.11; 95% CI: 0.01, 0.89; p for trend=0.122) (Figure 3A) and tryptophan (OR=0.10; 95% CI: 0.01, 0.87; p for trend=0.176) (Figure 3B) were negatively correlated with sarcopenic risk (Table 2).

The associations of amino acids with muscle mass function

By using GLM analysis, dietary intakes of lysine (p=0.011), threonine (p=0.022), leucine (p=0.025), valine (p=0.021), tryptophan (p=0.011), branched chain amino acids (p=0.032), and aromatic amino acids (p=0.033) were positively correlated with gait speed, respectively (Table 3).

DISCUSSION

This study is the first to investigate the associations of dietary protein and amino acid intakes with sarcopenic risk through a community-based study in participants over 65 years. The present study demonstrated that higher intakes of BCAAs were associated with a lower risk of sarcopenia. Additionally, BCAAs had significant benefits in improving the physical functions of the elderly. Meanwhile, appropriate protein intake (70 g) was associated with reduced risk of sarcopenia.

Based on recent cross-sectional surveys, the prevalence of sarcopenia of the present study (4.1%) was in the range of sarcopenia prevalence (0.4-13.9%).[13-17] Previous epidemiological studies have identified several factors affecting sarcopenia, including age, gender, BMI, physical

Table 1. Basic characteristics of subjects with and without sarcopenia

	n (%) or M (Q25, Q75) or Mean±SD†			p‡
	Total (n=1140)	Sarcopenia (n=47)	No sarcopenia (n=1093)	
Sarcopenia status, n (%)				
Sarcopenia	1093 (95.9)			
No sarcopenia	47 (4.12)			
Age, years	72.0 (68.0, 76.5)	82.0 (73.0, 86.0)	71.0 (68.0, 76.0)	<0.001
Gender, n (%)				0.022
Male	500 (43.9)	13 (27.7)	487 (44.6)	
Female	640 (56.1)	34 (72.3)	606 (55.4)	
Body height, cm	163 (157, 170)	156 (150, 160)	163.0 (158, 170)	<0.001
Body weight, kg	67.8 (60.3, 75.3)	51.3 (49.0, 55.7)	68.1 (61.4, 75.7)	<0.001
Body mass index, kg/m^2	25.5 (23.2, 27.6)	21.8 (19.8, 22.8)	25.7 (23.4, 27.7)	<0.001
Waist circumstance, cm	91.3±9.4	83.9±8.2	91.6±9.3	<0.001
Hip circumstance, cm	99 (94, 103)	92 (89.5, 95.5)	99 (95, 103)	<0.001
Waist hip ratio, %	0.92 (0.88, 0.96)	0.90 (0.85, 0.95)	0.92 (0.88, 0.96)	0.081
Blood pressure, mmHg				
Systolic pressure	136 (128, 148)	136 (125, 152)	136 (128, 147)	0.894
Diastolic pressure	78 (71, 85)	76 (71, 85)	78 (71, 85)	0.889
Marital status, n (%)				<0.001
Single	1 (0.09)	0 (0.00)	1 (0.10)	
Married	939 (86.8)	24 (60.0)	915 (87.8)	
Widowed	140 (12.9)	16 (40.0)	124 (11.9)	
Separated	2 (0.18)	0 (0.00)	2 (0.19)	
Education, n (%)				0.560
≤ Junior high school and others	662 (61.2)	27 (67.5)	635 (60.9)	
High school	267 (24.7)	7 (17.5)	260 (25.0)	
≥ Some college	153 (14.1)	6 (15.0)	147 (14.1)	
Current smoking, n (%)				0.759
Yes	119 (11.0)	5 (12.5)	114 (11.0)	
No	962 (89.0)	35 (87.5)	927 (89.1)	
Alcohol drinking, n (%)				0.007
Yes	202 (18.7)	1 (2.50)	201 (19.3)	
No	879 (81.3)	39 (97.5)	840 (80.7)	
Fasting blood glucose (mmol/L)	5.78 (5.22, 6.85)	5.62 (4.97, 7.02)	5.79 (5.23, 6.84)	0.219
TC (mmol/L)	5.91 (4.97, 6.84)	6.21 (5.49, 7.35)	5.89 (4.96, 6.81)	0.050
TG (mmol/L)	1.34 (0.92, 2.04)	1.07 (0.86, 1.69)	1.36 (0.92, 2.08)	0.023
LDL-C (mmol/L)	2.85 (2.33, 3.38)	3.02 (2.40, 3.57)	2.84 (2.32, 3.37)	0.138
HDL-C (mmol/L)	1.92 (1.60, 2.23)	2.04 (1.73, 2.42)	1.91 (1.60, 2.22)	0.036
Physical activity, n (%)				0.071
Low-impact exercise	403 (35.4)	24 (51.1)	379 (34.7)	
Moderate-impact exercise	328 (28.8)	10 (21.3)	318 (29.1)	
High-impact exercise	409 (35.9)	13 (27.7)	396 (36.2)	
Energy intake, kcal/d	1797 (1537, 2126)	1621 (1308, 1996)	1800 (1540, 2137)	0.026
Macronutrient intake				
Total protein, g/d	60.9 (47.8, 74.3)	51.1 (41.0, 65.1)	61.2 (48.2, 74.4)	0.013
Fat, g/d	75.4 (63.4, 90.8)	66.4 (59.6, 85.3)	75.9 (63.5, 90.9)	0.054
Carbohydrate, g/d	216 (173, 261)	186 (159, 237)	217 (173.9, 263)	0.031

HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; SD: standard deviation; TC: total cholesterol; TG: triglyceride.

†Data are presented as median (interquartile range) for continuous variables with non-normal distributions, as mean±SD for continuous variables with normal distributions or participants (percentage %) for categorical variables.

‡p for difference between groups was tested by Student's t-test, chi-square, and Wilcoxon rank sum test, respectively.

activity, dietary of energy and protein intakes.[25-27] Except the above-mentioned factors, this study also found that there were significant differences in height, weight, waist circumference, hip circumference, alcohol drinking, marital status and carbohydrate intake between healthy participants and sarcopenic patients. In addition, higher levels of serum TC and HDL-C, but lower TG levels might potentially increase in patients with sarcopenia. Regarding dietary factors, intake of dietary protein was a crucial factor associated with the risk of sarcopenia.

After adjusting for potential confounding factors, the risk of sarcopenia was significantly reduced when the protein intake ranged from 60.9 to 74.3 g/d. Among amino acids, dietary intakes of BCAAs, isoleucine and tryptophan were also negatively correlated with sarcopenic risk. It has been demonstrated that 15-20 grams of protein (7.5 g of essential amino acids) was sufficient to promote muscle protein synthesis in adult subjects. Compared with younger subjects, the elderly might need more protein to maintain muscle protein synthesis.[28] Santiago et al. reported that the protein intake of patients with sarcopenia was significantly lower than that of healthy participants.[29] In a cross-sectional study of the elderly in the Netherlands, a higher protein intake was associated with a 4% reduc-

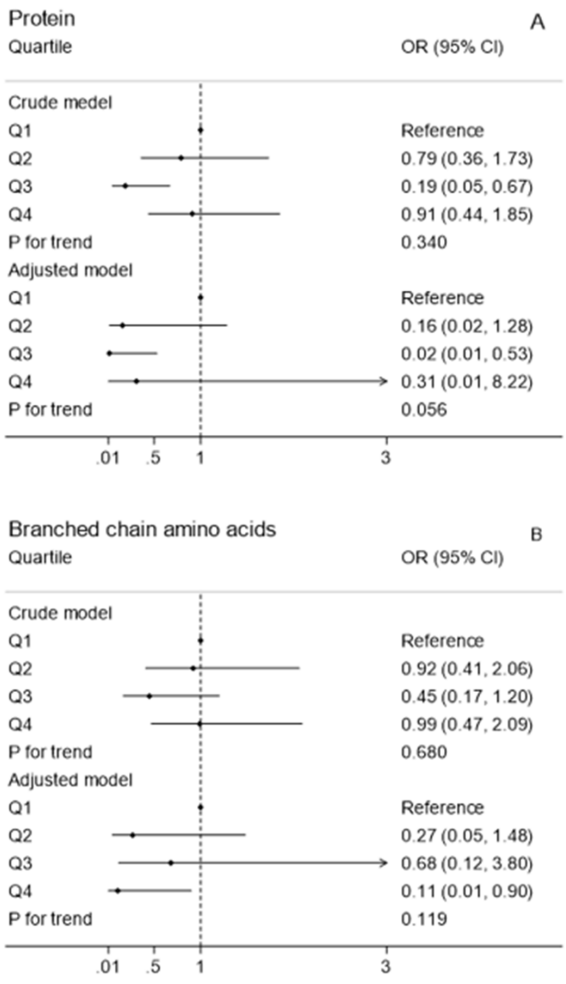

Figure 2. Association of dietary protein (A), branched chain amino acids (B) intakes with risk of sarcopenia.

tion in the incidence of sarcopenia.[30] A meta-analysis of randomized controlled trials study found that protein and amino acids supplementation exerted a positive impact on muscle mass in the elderly.[31] Supplemental 1-1.5 gram protein per kilogram bodyweight could prevent sarcopenia and maintain skeletal muscle mass in the elderly.[26,32,33] However, excessive protein intake (3 gram per kilogram) had no beneficial effect on muscle protein synthesis, potentially causing appetite loss,[34] tasting inhibition,[35] and resulting in impaired renal function in the elderly.[36] A previous study was consistent with the present results, indicating that appropriate protein intake might be beneficial in prevention of sarcopenia.

The relationships between dietary amino acid intakes and sarcopenic risk have received extensive attention. Amino acids generated by protein decomposition are the main essential amino acids to maintain muscle health in the elderly, especially BCAAs.[28,37] BCAAs (leucine, valine, and isoleucine) are the most abundant amino acids in proteins and can be exclusively obtained from dietary sources rather than endogenous synthesis. Meat, dairy products, eggs, beans, and cereals are rich in BCAAs, and these foods have important physiological roles in the regulation of protein synthesis, metabolism, food intake, and aging.[21] Le Couteur et al. summarized the effect of BCAAs supplementation on sarcopenia. They found that BCAAs supplementation alone was unlikely to be useful in sarcopenia; however, a diet rich in protein to increase the proportion of BCAAs had a positive effect on muscle mass in elders.[21] In an open-label clinical trial demonstrated that supplementation with BCAAs enriched mixture significant improved muscle mass and physical function in malnourished patients.[38] In addition, a randomized controlled trial showed that BCAAs supplementation (7.2 g/day) had positive effects on muscle strength and mass during a 5-week of intervention, but these positive effects were decreased after a 12-week of intervention.[39] Contrarily, animal model showed that supplementation with BCAAs or leucine contributed to increasing protein catabolism, while the weight of muscle remained unchanged.[40] Furthermore, several studies focused on the roles of BCAAs supplementation on muscle mass and function in patients with sarcopenia accompanied by other diseases.[21,28,38-47] Accordingly, the roles of BCAAs on muscle mass and functions were summarized and discussed in Table 4. In summary, BCAAs have beneficial effects on muscle mass and function. It is worth noting that when sarcopenia is combined with liver and kidney disease differs from sarcopenia alone, so the results of BCAAs supplementation in sarcopenia combined with liver and kidney disease should be interpreted with caution. Therefore, the effects of BCAAs on skeletal muscle mass and functions remain to be further investigated.

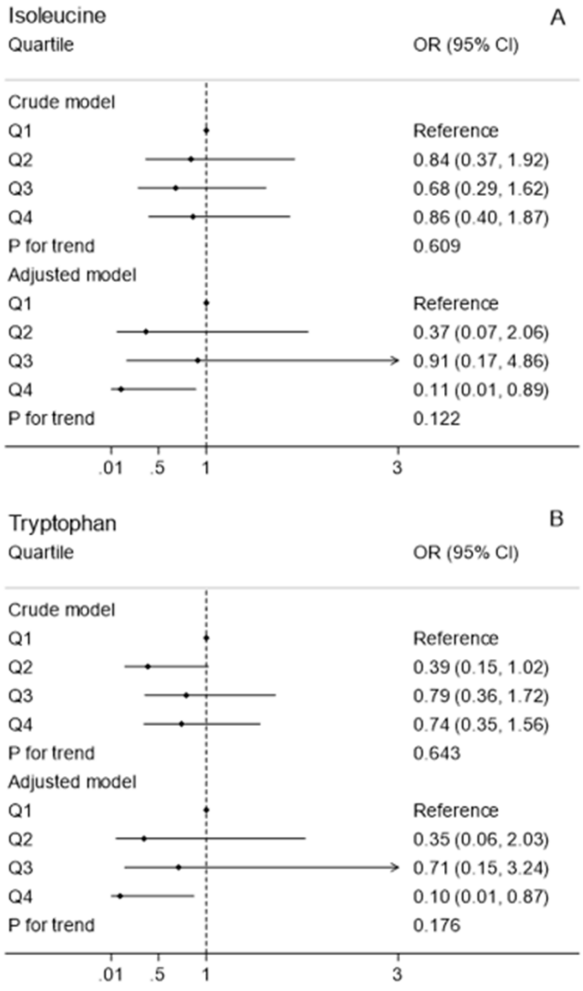

Figure 3. Association of dietary isoleucine (A), tryptophan (B) intakes with risk of sarcopenia.

Table 2. Multivariate adjusted odds ratios and 95% confidence intervals for sarcopenia compared to no sarcopenia by quartile of protein and amino acid intakes among 1140 elderly subjects[†]

	Q1	Q2	Q3	Q4	p[‡] for trend
Total protein, g/d	≤47.8	47.8-60.9	60.9-74.3	>74.3	
No. of sarcopenia/no sarcopenia	15/254	12/256	3/265	17/318	
OR (95% CI)	1.00 (reference)	0.79 (0.36, 1.73)	0.19 (0.05, 0.67)	0.91 (0.44, 1.85)	0.340
Multivariate adjusted OR (95% CI)	1.00 (reference)	0.16 (0.02, 1.28)	0.02 (0.01, 0.53)	0.31 (0.01, 8.22)	0.056
Branched chain amino acids, mg/d	≤3002.2	3002.2-4653.4	4653.4-6593.0	>6593.0	
No. of sarcopenia/no sarcopenia	13/256	12/256	6/262	16/319	
OR (95% CI)	1.00 (reference)	0.92 (0.41, 2.06)	0.45 (0.17, 1.20)	0.99 (0.47, 2.09)	0.680
Multivariate adjusted OR (95% CI)	1.00 (reference)	0.27 (0.05, 1.48)	0.68 (0.12, 3.80)	0.11 (0.01, 0.90)	0.119
Sulfur amino acids, mg/d	≤629.9	629.9-994.5	994.5-1417.5	>1417.5	
No. of sarcopenia/no sarcopenia	11/258	10/258	9/260	17/317	
OR (95% CI)	1.00 (reference)	0.91 (0.38, 2.18)	0.81 (0.33, 1.99)	1.26 (0.58, 2.73)	0.604
Multivariate adjusted OR (95% CI)	1.00 (reference)	0.71 (0.13, 3.79)	0.84 (0.16, 4.37)	0.35 (0.04, 3.09)	0.589
Aromatic amino acids, mg/d	≤1399.0	1399.0-2147.7	2147.7-3053.1	>3053.1	
No. of sarcopenia/no sarcopenia	13/256	12/256	6/262	16/319	
OR (95% CI)	1.00 (reference)	0.92 (0.41, 2.06)	0.45 (0.17, 1.20)	0.99 (0.47, 2.09)	0.682
Multivariate adjusted OR (95% CI)	1.00 (reference)	0.53 (0.10, 2.70)	0.76 (0.14, 4.14)	0.16 (0.02, 1.18)	0.190
Histidine, mg/d	≤436.0	436.0-675.5	675.5-960.3	>960.3	
No. of sarcopenia/no sarcopenia	13/256	10/258	8/261	16/318	
OR (95% CI)	1.00 (reference)	0.76 (0.33, 1.77)	0.60 (0.25, 1.48)	0.99 (0.47, 2.10)	0.873
Multivariate adjusted OR (95% CI)	1.00 (reference)	0.63 (0.13, 3.09)	0.71 (0.14, 3.60)	0.22 (0.03, 1.85)	0.287
Lysine, mg/d	≤1052.5	1052.5-1781.2	1781.2-2622.7	>2622.7	
No. of sarcopenia/no sarcopenia	14/255	7/261	10/259	16/318	
OR (95% CI)	1.00 (reference)	0.49 (0.19, 1.23)	0.70 (0.31, 1.61)	0.92 (0.44, 1.91)	0.977
Multivariate adjusted OR (95% CI)	1.00 (reference)	0.19 (0.03, 1.18)	1.21 (0.23, 6.38)	0.14 (0.02, 1.11)	0.283
Threonine, mg/d	≤672.2	672.2-1092.9	1092.9-1543.0	>1543.0	
No. of sarcopenia/no sarcopenia	14/255	11/257	6/263	16/318	
OR (95% CI)	1.00 (reference)	0.78 (0.35, 1.75)	0.42 (0.16, 1.10)	0.92 (0.44, 1.91)	0.565
Multivariate adjusted OR (95% CI)	1.00 (reference)	0.35 (0.07, 1.81)	1.15 (0.21, 6.20)	0.17 (0.02, 1.19)	0.217
Isoleucine, mg/d	≤717.5	717.5-1164.2	1164.2-1638.7	>1638.7	
No. of sarcopenia/no sarcopenia	13/256	11/257	299/260	14/320	
OR (95% CI)	1.00 (reference)	0.84 (0.37, 1.92)	0.68 (0.29, 1.62)	0.86 (0.40, 1.87)	0.609
Multivariate adjusted OR (95% CI)	1.00 (reference)	0.37 (0.07, 2.06)	0.91 (0.17, 4.86)	0.11 (0.01, 0.89)	0.122
Leucine, mg/d	≤1377.0	1377.0-2154.7	2154.7-2994.0	>2994.0	
No. of sarcopenia/no sarcopenia	13/256	10/258	8/260	16/319	
OR (95% CI)	1.00 (reference)	0.76 (0.33, 1.77)	0.61 (0.25, 1.49)	0.99 (0.47, 2.09)	0.858
Multivariate adjusted OR (95% CI)	1.00 (reference)	0.25 (0.04, 1.48)	0.61 (0.12, 3.15)	0.13 (0.01, 1.13)	0.212

CI: confidence interval; No.: number of subjects; OR: odds ratio.

[†]The multivariable-adjusted model of logistic regression was adjustment for age, gender, height, weight, BMI, waist circumference, hip circumference, waist hip ratio, blood pressure, marital status, education level, smoking, alcohol drinking, physical activity, fasting blood glucose, TC, TG, HDL-C, LDL-C, daily energy intake, carbohydrate intake, fat intake.

[‡]p for trends were conducted by assigning the median value for each category and modelling this variable as a continuous variable.

Table 2. Multivariate adjusted odds ratios and 95% confidence intervals for sarcopenia compared to no sarcopenia by quartile of protein and amino acid intakes among 1140 elderly subjects[†] (cont.)

	Q1	Q2	Q3	Q4	p[‡] for trend
Valine, mg/d	≤877	877-1360	1360-1910	>1910	
No. of sarcopenia/no sarcopenia	14/254	10/259	7/262	16/318	
OR (95% CI)	1.00 (reference)	0.70 (0.31, 1.61)	0.48 (0.19, 1.22)	0.91 (0.44, 1.91)	0.646
Multivariate adjusted OR (95% CI)	1.00 (reference)	0.53 (0.11, 2.63)	0.61 (0.12, 3.19)	0.16 (0.02, 1.16)	0.145
Tryptophan, mg/d	≤243	243-379	379-533	>533	
No. of sarcopenia/no sarcopenia	15/254	6/262	12/257	14/320	
OR (95% CI)	1.00 (reference)	0.39 (0.15, 1.02)	0.79 (0.36, 1.72)	0.74 (0.35, 1.56)	0.643
Multivariate adjusted OR (95% CI)	1.00 (reference)	0.35 (0.06, 2.03)	0.71 (0.15, 3.24)	0.10 (0.01, 0.87)	0.176

CI: confidence interval; No.: number of subjects; OR: odds ratio.
[†]The multivariable-adjusted model of logistic regression was adjustment for age, gender, height, weight, BMI, waist circumference, hip circumference, waist hip ratio, blood pressure, marital status, education level, smoking, alcohol drinking, physical activity, fasting blood glucose, TC, TG, HDL-C, LDL-C, daily energy intake, carbohydrate intake, fat intake.
[‡]p for trends were conducted by assigning the median value for each category and modelling this variable as a continuous variable.

Table 3. Prediction of the relationships between dietary intakes of protein and amino acids and muscle mass functions by generalized linear regression[†]

	Grip strength				Gait speed			
	Crude model		Adjusted model		Crude model		Adjusted model	
	β (SE)	p	β (SE)	p	β (SE)	p	β (SE)	p
Total protein, g/d	0.12 (0.01)	<0.001	-0.02 (0.02)	0.343	0.00 (0.00)	<0.001	0.00 (0.00)	0.054
BCAAs, g/d	0.57 (0.09)	<0.001	-0.11 (0.09)	0.186	0.01 (0.00)	<0.001	0.01 (0.00)	0.032
Sulfur amino acids, g/d	2.52 (0.44)	<0.001	-0.40 (0.38)	0.289	0.05 (0.01)	<0.001	0.02 (0.01)	0.203
Aromatic amino acids, g/d	1.23 (0.20)	<0.001	-0.21 (0.18)	0.262	0.02 (0.00)	<0.001	0.01 (0.01)	0.033
Histidine, g/d	4.07 (0.64)	<0.001	-0.75 (0.60)	0.214	0.08 (0.02)	<0.001	0.04 (0.02)	0.056
Lysine, g/d	1.24 (0.21)	<0.001	-0.28 (0.20)	0.171	0.03 (0.01)	<0.001	0.02 (0.01)	0.011
Threonine, g/d	2.27 (0.38)	<0.001	-0.52 (0.36)	0.151	0.05 (0.01)	<0.001	0.03 (0.01)	0.022
Isoleucine, g/d	2.19 (0.36)	<0.001	-0.38 (0.33)	0.253	0.04 (0.01)	<0.001	0.02 (0.01)	0.084
Leucine, g/d	1.21 (0.20)	<0.001	-0.25 (0.19)	0.176	0.02 (0.00)	<0.001	0.01 (0.01)	0.025
Valine, g/d	1.96 (0.32)	<0.001	-0.42 (0.30)	0.168	0.04 (0.01)	<0.001	0.02 (0.01)	0.021
Tryptophan, g/d	7.75 (1.20)	<0.001	-0.77 (1.09)	0.481	0.14 (0.03)	<0.001	0.09 (0.04)	0.011

BCAAs: branched chain amino acids; SE: standard error.
[†]The multivariable-adjusted model of generalized linear analyses was adjustment for age, gender, height, weight, BMI, waist circumference, hip circumference, waist hip ratio, blood pressure, marital status, education level, smoking, alcohol drinking, physical activity, fasting blood glucose, TC, TG, HDL-C, LDL-C, daily energy intake, carbohydrate intake, fat intake.

Table 4. Effect of Branched chain amino acids (BCAAs) on muscle mass and functions

Author, Year	Country	Type of article	Conclusions/ Evidence	Effect on muscles
Buondonno, 2020[38]	Italy	Open-label Randomized Trial	Supplementation with BCAAs enriched mixture significant improved muscle mass and physical function in malnourished patients.	Positively
Dasarathy, 2016[47]	Italy	Review Article	BCAAs improved protein synthesis and improve muscle mass by inhibiting the amino acid deficiency sensor, GCN2 and reversing eIF2α phosphorylation.	Positively
Hanai, 2015[41]	Japan	Retrospective Study	BCAAs supplementation were associated with improved survival of sarcopenic patients with liver cirrhosis.	Positively
Hanai, 2017[46]	Japan	Retrospective Study	Decreasing serum BCAAs levels was associated with sarcopenia, and BCAAs supplementation may be one of the therapeutic options for sarcopenia and minimal hepatic encephalopathy in patients with liver cirrhosis.	Positively
Hiraoka, 2017[45]	Japan	longitudinal Study	BCAAs supplementation and walking exercise were found to be effective and easily implemented for improving muscle volume and strength in liver cirrhosis patients.	Positively
Holecek, 2016[40]	Czech Republic	Animal Trial	The results failed to demonstrate positive effects of the chronic consumption of BCAAs or leucine-enriched diets on protein balance in skeletal muscle.	Negatively
Kitajima, 2018[42]	Japan	longitudinal Study	In patients with liver cirrhosis, supplementation with BCAAs were related to decreased fat accumulation in skeletal muscle, which maintained skeletal muscle mass and ameliorated glucose tolerance.	Positively
Ko, 2020[39]	China	Single-arm Intervention Study	Supplementation with enriched BCAAs for 5 weeks correlates with short-term positive effects on sarcopenic parameters but attenuation of those effects following discontinuation for 12 weeks.	Positively
Le Couteur, 2020[21]	Australia	Review Article	BCAAs supplements by themselves are unlikely to be useful in sarcopenia, however, BCAAs as part of higher protein intake, enriched amino acid supplement or a protein supplement are associated with improvements of muscle function in older people.	Neutrally
Nishikawa, 2016[44]	Japan	Review Article	Decreased BCAAs concentration in the blood and/or muscles could lower ammonia clearance from the blood, and lead to both the progression of hepatic encephalopathy and the increase of the severity of sarcopenia.	Positively
Rondanelli, 2015[28]	Italy	Review Article	At rest, BCAAs, in particular leucine, have an anabolic effect by an increasing protein synthesis and/or a reducing the rate of protein degradation, resulting in a positive net muscle protein balance.	Positively
Sinclair, 2016[43]	Australia	Review Article	In sarcopenic cirrhotic patients, both reduced circulating BCAAs levels and reduced muscle mass might contribute to impaired ammonia clearance.	Positively

BCAAs: branched chain amino acids; GCN2: general control nondepressed 2; eIF2α: eukaryotic initiation factor 2 α.

It is necessary and significant to supplemental essential amino acids contributing to skeletal muscle anabolism.[48] The positive effect of BCAAs on muscle is achieved by promoting protein synthesis and reducing protein degradation (Figure 4).[20] BCAAs stimulate muscle anabolism by promoting mTOR phosphorylation. With the activation of P70S6K, a downstream of mTOR, it increases protein synthesis through activating S6 and eIF4B.[49] Meanwhile, it has been reported that BCAAs could reduce the Atrogin-1 and MuRF-1 mRNA expression mediated by mTOR.[50] Atrogin-1 and MuRF-1 are E3 ubiquitin ligases expressed in skeletal muscle. Reduced levels of these two substances could decrease the ubiquitination of protein and reduce the degradation of muscle protein.[51] Isoleucine might have synergies with insulin to activate mTOR to reduce the loss of muscle mass or physical function.[21] Besides, one study found that tryptophan increased the lipid peroxidation of muscle by inducing the kynurenine pathway, thereby increasing the risk of muscle reduction.[52] But this mechanism is contrary to our findings. Further research is needed to explore the relationship between a single amino acid and sarcopenia.

The associations of dietary protein and amino acid intakes with muscle mass functions were also investigated. To date, no epidemiological study has reported the relationships between individual amino acids and muscle mass functions. A double-blind randomized controlled trial with 6 months of intervention showed that supplemental protein with a new chicken oral liquid significantly improved the gait speed in the higher-level physical activity group in the elderly over 70 years, compared with controls.[53] Meanwhile, BCAAs supplementation could improve muscle function in patients with sarcopenia in short period of intervention, but not for a long period of intervention.[39] Regarding the relationship between single amino acid and muscle mass function, tryptophan metabolites were associated with the increased risk of sarcopenia,[54,55] but another study showed that serum kynurenine levels (a tryptophan metabolite) were negatively associated with grip strength and gait speed.[52] Besides, no study has explored the effects of dietary aromatic amino acids, lysine and threonine supplementation on muscle mass function, and that needs further research.

There are several advantages of this study to highlight. To the best of our knowledge, this study is the first to explore the relationships between dietary intakes of protein and amino acids and sarcopenic risk. The associations of protein and amino acids with muscle mass functions have also been investigated. Secondly, the present community-based study provided strong evidence that appropriate protein intake with higher BCAAs intakes was inversely associated with risk of sarcopenia. We believe that the findings of this study have public health significance for the prevention of sarcopenia. They also deepen our understanding of the associations between BCAAs and sarcopenia. There are limitations of this study. Firstly, the accuracy of dietary protein and amino acid intakes is crucial for component-based epidemiological study, but uncertain. Although the validated 3-day food records were used to evaluate the dietary intakes of protein and amino acids, measurement error was inevitable in participants over 65 years. Secondly, epidemiological research is inevitably limited by potentially unrecognized confounding factors. The multivariable-adjusted models have inherent bias and residual confounding factors attributable to inaccurate or unmeasured risk factors which might affect the final relationships advanced.[24]

Conclusion

In conclusion, the present study demonstrates that a moderate increase in protein intake is associated with a reduced risk of sarcopenia. Furthermore, higher intakes of BCAAs are associated with reduced risk of sarcopenia and improved physical functions. We believe the findings of the present study have significant public health relevance for the prevention of sarcopenia. To confirm the

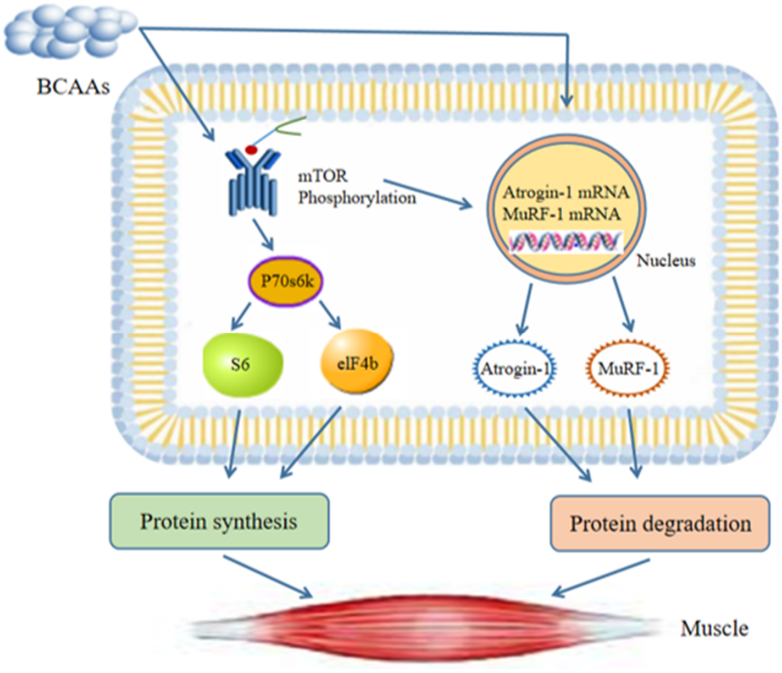

Figure 4. The mechanism of branched chain amino acids on muscle by affecting protein synthesis and degradation pathway.

findings of this study, high-quality epidemiological studies with larger sample-size are warranted to be implemented in other regions and ethnic origins.

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AUTHOR DISCLOSURES

The authors declare this work has no conflict of interest.

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Original Article

Nutritional complexity in children with ADHD related morbidities in China: A cross-sectional study

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Background and Objectives: To assess the general and nutritional health of children with attention deficit/hyperactivity disorder (ADHD). **Methods and Study Design:** The National Multicenter Sleep Research Database for 23791 school-age children in grades 1-6 from 9 cities in China was accessed. Children with a specialist diagnosis of ADHD or not (non-ADHD) in 2005 were studied. National anthropometric growth standards for children aged 2-18 years classified children as underweight, wasted, stunted (short stature presumed nutritional), or overweight/obesity. Independent variables were preterm birth, sleep quality and prior disease and ADHD was the dependent variable. Binary logistic regression models were developed along with interaction analyses for associated disorder or disease on overweight/obesity, and stunted. **Results:** Some 18731 records were analyzed for 808 children with ADHD. The comparative prevalences for ADHD with non-ADHD children were stunted 9.8% vs 5.9% (p<0.001) and overweight/ obesity (32.6% vs 29.6%, p=0.002) respectively. ADHD boys were more often underweight (7.5% vs 5.3%, p=0.027), but not in girls. ADHD likelihood Odds Ratios, ORs (with 95%CI) were for premature birth 1.838, (1.393-2.423), allergic diseases 1.915 (1.526-2.399), otitis media 1.54 (1.118-2.146), tonsillar or adenoid hypertrophy1.662 (1.348-2.050), gastroesophageal reflux 3.008(1.792-1.792-5.049), and sleep disorder 2.201(1.847-2.623) were ADHD risk factors. Only poor sleep quality and ADHD exhibited an interaction for stunted with OR=0.409 (0.233-0.719). **Conclusions:** Compromised and complex nutritional health in ADHD children challenges clinical nutrition with a range of health problems, albeit coherent with the needed nutritional emphasis in the 'first 1000 days'.

Key Words: attention deficit disorder with hyperactivity, stunted, underweight, overweight/obesity, school-age children, first 1000 days

INTRODUCTION

Attention deficit/hyperactivity disorder (ADHD) is the most common neurodevelopmental disorder affecting the health of children and adolescents. The incidence of ADHD in China is 5.7%.[1] ADHD is a chronic disorder as 60-80% of ADHD cases can persist through adolescence, and 50% are affected in adulthood, with 71.9% of ADHD patients having concomitant disease.[2] The direct or potentially synergistic effects of ADHD, with comorbidities and pharmacotherapy, increase proneness to further health problems. These include nutritional disorders of growth, energy regulation attributable to physical activity, eating disorders,[3] sleep disorders or poor diet,[4,5] and immunoinflammatory disorder such as recurrent infection and allergic disease.[6-9] In children with ADHD who have reduced height and growth rates this may have been due to the long-term use of stimulants.[7] To understand, recognise and more effectively manage the health status of school-age children with ADHD in China, their physical growth and disease status the national multicenter children's sleep quality survey database. Our general hypothesis is that growth and body composition contribute to the health and wellbeing of children with ADHD, implicitly, to its mitigation and management.

METHODS

Participants and study design

Cluster sampling was conducted from November to December 2005 in 32 provinces (cities and autonomous regions). Nine cities including Shanghai, Guangzhou, Xi'an, Wuhan, Chengdu, Harbin, Hohhot, Urumqi and Shi he zi, were randomly selected for the questionnaire survey.

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Cluster-stratification was applied by city, district, school, grade, and class. Finally, 39 districts and 55 primary schools were selected (Figure 1). The study was approved by the Ethics Committee of Xinhua Hospital Affiliated with Shanghai Jiaotong University (No. XHEC-D-2017-047).

Questionnaires

Study objectives were explained to the principals and teachers of the selected schools. After permission was granted, children were asked to take the proposal, questionnaire and explanatory files to their parents. Participation depended on parental agreement and an assurance that information provided would be treated with anonymity. Eligible students were aged 5-12 years (grades 1 to 6) and studying in the selected schools. The survey was in three parts. The first comprised key sociodemographic information including gender, age, and perinatal factors (delivery mode, gestational weeks, and feeding patterns). The second documented family environmental factors (education level of the caregivers, family income, marital status of parents, and geographic location) The third explored disease history (ADHD, nasal obstruction, asthma, otitis media, tonsillar or adenoid hypertrophy, gastroesophageal reflux, food allergy, allergic rhinitis, and obesity). Each questionnaire had an informed consent form attached for agreement.

Measurement of physical growth

Physical measurements were made by school doctors and community health centers. Weight was measured using an electronic pediatric scale to the nearest 0.1 kg, and length was measured using a pediatric ruler to the nearest 0.1 cm. Growth and body compositional status was classified by the criteria for 2- to 18-year-old children in China in 2005, according to sex and age. Children below the 3rd percentile for weight were defined as underweight, below the 3rd percentile value of height as stunted, below the 3rd percentile value of body mass index (BMI) as wasted, above the 85th percentile value of BMI as overweight, and above the 97th percentile of BMI as obese.[10]

ADHD diagnosis

ADHD presence was based firstly on the parents' answers to the question: "has the child been diagnosed with ADHD by a specialist (developmental behavioral pediatrician or psychiatrist, DSM-IV diagnostic standard)? (yes or no)." Then, children were divided into an ADHD group and a non-ADHD group according to whether ADHD was diagnosed by a specialist. Behavioral–developmental pediatricians at behavioral pediatric clinics made ADHD diagnoses using the Diagnostic and Statistical Manual of Mental Disorders–Fourth Edition (DSM-IV; American Psychiatric Association, 2000) diagnostic criteria.

Age groups

The age range was from 6 years 0 days to 12 years 11-30 days, and each age group divided further into the following groups. Six years 0 days to 6 years 11-30 days as the 6-year-old group, and this pattern followed through to the 12-year-old group, group, resulting in 6 groups: the 6-, 7-, 8-, 9-, 10-, 11- and 12-year-old groups. This grouping avoided interference by children under 6 years old and over 13 years old (possible because of the early school and follow-up school children populations).

Statistical analysis

SPSS 21.0 (IBM, Armonk, NY, USA) was used for statistical analysis. Categorical data were described as frequencies and analyzed by chi-square. Continuous data are shown as mean ± standard deviation and analyzed with Student's t test or analysis of variance with Tukey's post hoc test, as appropriate. The factors with statistical significance on univariable analysis were included as independent factors in the multivariable binary logistic regression model along with ADHD as the dependent factor. Using preterm birth, sleep quality and prior disease as independent variables and diagnosed ADHD as the dependent variable, binary logistic regression models were established. Difference was regarded as statistically significant with $p<0.05$.

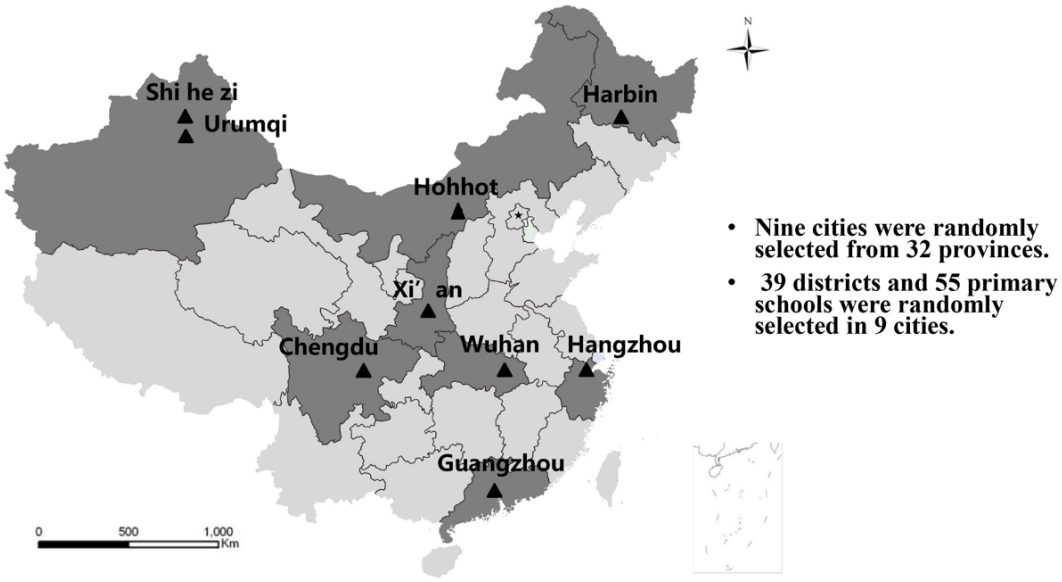

Figure 1. Geographical distribution of the nine cities (shaded for corresponding province) in China and number of selected schools.

RESULTS

Malnutrition of school-age ADHD children and non-ADHD children

In the survey, 23,791 children recruited from six grades of the chosen schools, 22018 (92.5%) returned completed questionnaires, and 970 (4.4%) children with ADHD. Among them, 18731 [9252 boys (49.4%) and 9479 girls (50.6%)] students within 6-12 years completed physical measurements, and 808 (4.3%) children with ADHD [532 boys (6.6%) and 276 girls (3.4%)], met the inclusion criteria.

Overweight, obesity, wasted, underweight and stunted prevalences were 15.9%, 11.9%, 4.8%, 2.5% and 5.2%, respectively. As shown in Table 1, the proportion of ADHD children who were overweight and obese was significantly higher than for non-ADHD children (32.6% vs 29.6%, χ^2=9.904, p=0.002). Those wasted among ADHD children were more prevalent than among non-ADHD children, but there was no significant differences between these groups (7.1% vs 5.5%, χ^2=3.461, p=0.063). The prevalence of stunted was significantly higher among ADHD children than among non-ADHD children (9.8% vs 5.9%, χ^2=20.353, p<0.001). There were no significant differences for underweight between the two groups (3.0% vs 2.9%, χ^2=0.009, p=0.924).

Sex difference in malnutrition among school-age ADHD children and non-ADHD children

The prevalence of stunted, underweight, being wasted and overweight/obesity in boys with ADHD were 9.6%, 3.4%, 7.5% and 35.2%, respectively.

As shown in Table 1, the stunted prevalence was higher in boys with ADHD than in boys without ADHD (9.6% vs 5.7%, χ^2=13.389, p<0.001), and being wasted was also more common in boys with ADHD (7.5% vs 5.3%, χ^2=4.877, p=0.027). For underweight and overweight/obesity, there were no significant differences between groups (underweight, 3.4% vs 2.9%, χ^2=0.389, p=0.533; overweight/obesity, 35.2% vs 35.4%, χ^2=0.018, p=0.894).

In girls, only being of shorter stature was higher with ADHD than in non-ADHD girls (10.1% vs 6.1%, χ^2=7.644, p=0.006). There were no significant differences in prevalence for underweight, being wasted or overweight/obesity between groups (Table 1, Figure 2).

Nutritional status of ADHD and non-ADHD children by age

As shown in Table 2 (Figure 2), except for the 7-year-old age group, the prevalence of being wasted was higher in ADHD than in non-ADHD students (10.53% vs 4.83%, χ^2=7.450, p=0.006). There was no statistically significant differences in prevalence for underweight, being wasted or overweight/obesity in the other age groups of ADHD and non-ADHD children. While the prevalence of overweight/obesity in ADHD children is significantly different in the total population, it is not statistically significant by age group.

In ADHD children stunted prevalence was higher than that in non-ADHD children in the 7-year-old group (10.26% vs 5.15%, χ^2=6.079, p=0.014), in the 8-year-old group (8.88% vs 5.03% χ^2=4.383, p=0.036), in the 9-year-old group (10.73% vs 5.96%, χ2=7.224, p=0.007) and in the 10-year-old group (8.82% vs 5.02%, χ^2=4.427, p=0.035). There was no significant difference in the prevalence of stunted in the 11- to 12-year-old groups between ADHD and non-ADHD children.

Multivariate logistic regression analysis of risk factors for ADHD

As shown in Table 3, a multivariable analysis was deployed to investigate risk factors for ADHD among school-age children. Adjustments were made for individual factors (gender, age), perinatal factors (delivery mode, feeding mode), and family environment factors (according to the education level of the guardian, per capita income of the family, marital status of parents and geographical distribution). Preterm birth (OR=1.838, 95% CI: 1.393-2.423), allergic diseases (allergic rhinitis, food allergy, asthma and repeated nasal congestion) (OR=1.915, 95% CI: 1.526-2.399), otitis media (OR=1.549, 95% CI: 1.118-2.416), tonsil or adenoid hypertrophy (OR =1.662, 95% CI: 1.348-2.050), gastroesophageal reflux (OR=3.008, 95% CI: 1.792-5.049), and poor sleep quality (OR=2.201, 95% CI: 1.847-2.623)
were risk factors for ADHD.

Table 1. Stunted, underweight, wasted, overweight/obesity prevalences for ADHD and non-ADHD school age children (2005 growth references for Chinese children aged 2-18 years)

	Total		Boys		Girls	
	ADHD (n=808)	Non-ADHD (n=17923)	ADHD (n=532)	Non-ADHD (n=8720)	ADHD (n=276)	Non-ADHD (n=9203)
Stunted, n (%)	79 (9.8)	1058 (5.9)	51 (9.6)	499 (5.7)	28 (10.1)	559 (6.1)
χ^2	20.353		13.389		7.644	
p	<0.001***		<0.001***		0.006**	
Underweight, n (%)	24 (3.0)	522 (2.9)	18 (3.4)	254 (2.9)	6 (2.2)	268 (2.9)
χ^2	0.009		0.389		0.520	
p	0.924		0.533		0.471	
Wasted, n (%)	57 (7.1)	989 (5.5)	40 (7.5)	461 (5.3)	17 (6.2)	528 (5.7)
χ^2	3.461		4.877		0.088	
p	0.063		0.027*		0.767	
overweight/obesity, n (%)	264 (32.6)	4947 (29.6)	187 (35.2)	3090 (35.4)	77 (27.9)	2233 (24.3)
χ^2	9.904		0.018		1.921	
p	0.002*		0.894		0.166	

n: Number;.* p<0.05; *** p<0.01; *** p<0.001

Table 2. Stunted, underweight, wasted, overweight/obesity prevalences for ADHD and non-ADHD school-age children by age group (2005 growth reference for Chinese children aged 2-18 years)

Age (year-old)	Number		Stunted (%)		Underweight (%)		Wasted (%)		Overweight/obesity (%)	
	ADHD	Non-ADHD	ADHD	Non-ADHD	ADHD	Non-ADHD	ADHD	Non-ADHD	ADHD	Non-ADHD
6~	70	1961	8.82	4.01	1.41	1.53	7.35	4.65	35.29	30.87
χ^2			3.338		0.007		1.066		0.600	
p			0.068		0.935		0.302		0.438	
7~	115	3136	10.26	5.15	0.00	1.82	10.53	4.83	35.96	33.23
χ^2			6.079		2.128		7.450		0.371	
p			0.014		0.145		0.006		0.542	
8~	165	3217	8.88	5.03	4.62	3.11	8.33	5.96	27.38	29.76
χ^2			4.383		1.223		1.569		0.431	
p			0.036		0.269		0.210		0.511	
9~	174	3115	10.73	5.69	2.79	2.31	3.98	5.55	31.82	27.22
χ^2			7.224		0.172		0.801		1.767	
p			0.007		0.678		0.371		0.184	
10~	130	3023	8.82	5.02	1.52	2.25	4.55	5.38	28.03	26.82
χ^2			4.427		0.314		0.173		0.094	
p			0.035		0.575		0.677		0.760	
11~	94	2375	7.37	6.50	1.05	4.29	8.42	6.19	25.26	21.43
χ^2			0.117		2.403		0.769		0.795	
p			0.732		0.121		0.380		0.373	
12~	38	1097	10.00	9.09	11.90	5.47	10.00	6.14	20.00	18.70
χ^2			0.008		3.113		0.977		0.043	
p			0.928		0.078		0.323		0.836	

Table 3. Likelihood of health disorder in school age children with ADHD by multivariate logistic regression analysis

Factor	ADHD n (%)	Non-ADHD n (%)	β (SE)	OR†	95% CI
preterm birth	70 (8.7)	985 (5.5)	0.608 (0.141)	1.838	1.393-2.423
allergic diseases	211 (26.1)	2724 (15.2)	0.650 (0.115)	1.915	1.526-2.399
otitis media	54 (6.7)	681 (3.8)	0.437 (0.166)	1.549	1.118-2.146
tonsil or adenoid hypertrophy	162 (20.0)	1989 (11.1)	0.517 (0.100)	1.662	1.348-2.050
gastroesophageal reflux	35 (4.3)	108 (0.6)	1.101 (0.264)	3.008	1.792-5.049
poor sleep quality	555 (68.7)	8442 (47.1)	0.789 (0.089)	2.201	1.847-2.623

n: Number.
†Adjusted for age and gender, perinatal factors (mode of delivery and feeding) and family environmental factors (geographical distribution, economic status, caregiver culture, parental marital relationship).

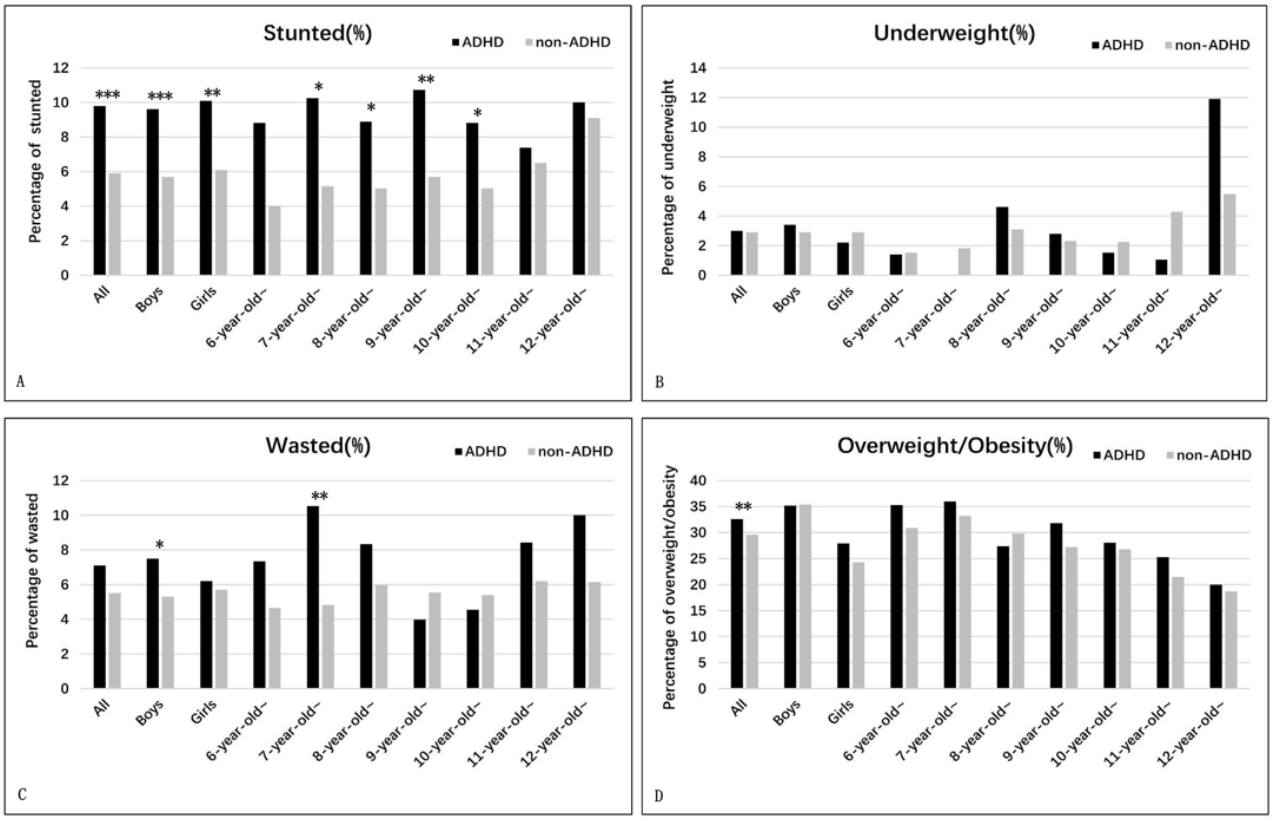

Figure 2. The percentage of (A) stunted, (B) underweight, (C) wasted and (D) overweight/obesity from 2 to 12-year-old school age children with or without ADHD. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

As shown in Table 4 and Figure 3, interaction analyses were undertaken to investigate the effects of ADHD and associated disorder or diseases on overweight/obesity and stunted. Only poor sleep quality and ADHD exhibited significant interaction (OR=0.409, 95% CI: 0.233-0.719).

DISCUSSION

ADHD is recognized a major psychological disorder among children and adolescents. It affects growth and development as confirmed in the present investigation, but the mechanisms and broader consequences are unclear. In China, the focus of guidelines for diagnosis and management is behavioural and pharmacotherapy,[2,9] with little attention to the potential of nutritional management.

We found that ADHD children were more commonly of both shorter stature and overweight/obese, representing disordered growth and body composition. This phenomenon is seen increasingly with economic disparity and development, and referred to as the' double burden of disease', an undoubted oversimplification of its pathogenesis.[12-14] The proportion stunted was high in the 7- to 9-year-old age group, the main age range for the diagnosis and prescription of medication for ADHD children. Risk factors for and co-morbidities with ADHD were found to include preterm birth, allergic disease, otitis media, tonsillar or adenoid hypertrophy, gastroesophageal reflux and poor sleep quality. The nutritional status of school-age children with ADHD in China is in a state of polarization, which suggests not only should be paid attention to drug and behavior therapy, but nutrition management should also be included in the development of ADHD diagnosis and treatment guidelines. The finding of an

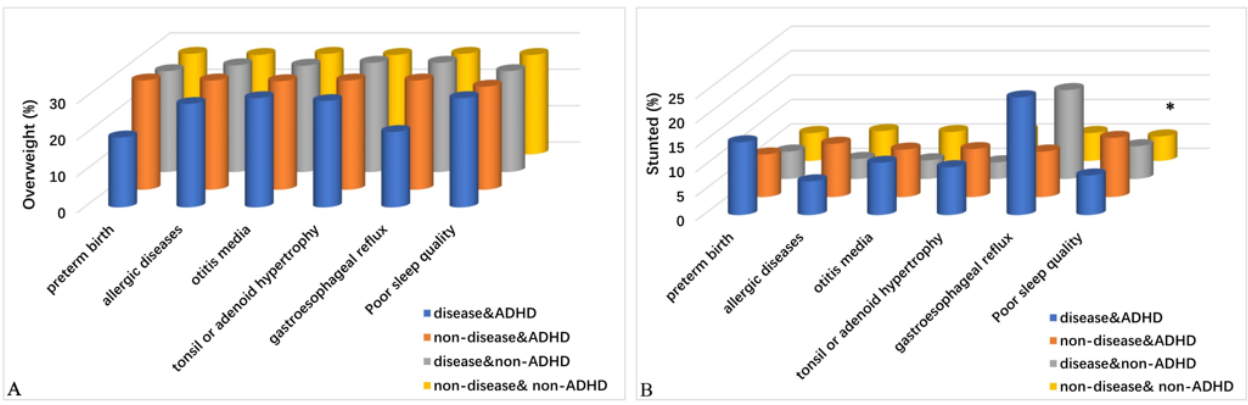

Figure 3. (A) Overweight/obesity and (B) stunted prevalence with or without ADHD & associated disorder or disease. $^{*}p<0.05$.

Table 4. Interactions of ADHD with health status for overweight/obesity or stunted by binary logistic regression analysis

Factor	Overweight/obesity n (%)					Stunted n (%)				
	ADHD	Non-ADHD	*p*	OR[†]	95% CI	ADHD	Non-ADHD	*p*	OR[‡]	95% CI
Preterm birth										
Yes	14 (19.2)	258 (27.7)	0.055	0.539	0.286-1.014	11 (14.9)	54 (5.6)	0.143	1.848	0.812-4.202
No	216 (30.7)	4593 (27.6)				62 (8.7)	967 (5.7)			
Allergic diseases										
Yes	61 (28.4)	810 (29.3)	0.509	0.875	0.589-1.300	15 (6.9)	116 (4.1)	0.553	0.801	0.385-1.666
No	177 (30.5)	4139 (27.3)				64 (10.9)	941 (6.1)			
Otitis media										
Yes	18 (32.1)	202 (29.2)	0.660	0.861	0.441-1.680	6 (10.7)	27 (3.8)	0.275	0.533	0.173-1.647
No	221 (29.8)	4748 (27.6)				73 (9.7)	1031 (5.9)			
Tonsil or adenoid hypertrophy										
Yes	47 (29.2)	620 (30.3)	0.325	1.246	0.804-1.929	16 (9.8)	70 (3.4)	0.167	0.591	0.281-1.246
No	192 (30.2)	4330 (27.3)				63 (9.8)	988 (6.1)			
Gastroesophageal reflux										
Yes	6 (20.7)	35 (32.4)	0.166	2.185	0.723-6.602	7 (24.1)	20 (18.2)	0.410	1.719	0.474-6.233
No	233 (30.3)	4914 (27.6)				72 (9.3)	1037 (5.7)			
Poor sleep quality										
Yes	150 (30.1)	2161 (27.7)	0.206	1.146	0.787-1.669	41 (8.1)	533 (6.7)	0.002	0.409	0.233-0.719
No	60 (28.3)	2391 (27.3)				27 (12.1)	441 (5.0)			

[†]OR: Interaction analysis by binary logistic regression for overweight/obesity as the dependent factor.
[‡]OR: Interaction analysis by binary logistic regression for stunted as the dependent factor.

association of ADHD with premature birth indicates that affected children were likely to have been born with a low birth weight and its attendant intrauterine nutritional determinants and long-term consequences as with intrauterine growth retardation (IUGR).[15,16] This situation is coherent with the substantial evidence that the first 1000 days from conception require attention to their nutritional optimisation for healthful human development.[17,18] Long-term clinical nutrition management in children with ADHD commences pre-conception with maternal health and continues through pregnancy to infancy and childhood. Mitigation in childhood will take account of several potential exacerbators: (1) proneness to abnormal eating behavior (2) nutrition- drug interactions and compliance; and (3) a spectrum of nutritional disorders and interactive complexity.

The probable association of ADHD with abnormal eating behavior, partly manifest in disordered growth (shorter stature) and body composition (fatness and its distribution) has been anticipated in earlier studies in China that of Yang et al conducted in 2007 in the Guangxi area is consistent with the present study.[19] In 2010,[20] Zhejiang reported that the overweight/obesity prevalence among 158 children with ADHD was 29.1%, higher than the 14.6% among children in the Family Planning Commission's investigation of student physique and health and where the basis of comorbidity is considered to be principally abnormal dietary pattern behavior. ADHD sufferers have diminished alertness and an increased prevalence of sleep disorders, with the greater likelihood of energy dense food choice, notably that of fast food, rather than healthful alternatives and those prepared by their own families.[21] Moreover, the Decreased alertness results in a delay in food satisfaction, disordered eating, such as not eating breakfast,[21] overeating, more snacking , sedentary behaviour and excessive screen time.[22-24] ADHD can also be associated with 'stress overeating',[25] a further contributor to overfatness in ADHD.

That ADHD may be familial, although not necessarily genetic, is of interest on account of shared and transmitted socioeconomic circumstances and behaviours, even with epigenetic expression.[26-28] In 2017,[29] Chen et al conducted a family aggregation study on ADHD prevalence, education, comorbidities (including drug abuse, anxiety, depression) and the associated overweight/obesity prevalences among Swedish servicemen and their offspring, finding that overfatness and ADHD have a shared familial risk whose specification is unclear. Insofar as genomic explanations are concerned, the overweight obesity and ADHD comorbidity may depend on such as the rs805013 fragment in FTO (obesity gene) as a mediator of the pathogenesis of ADHD.[30] The DRD4 gene is also involved in the pathogenesis of ADHD and increases the risk of obesity.[31] Low-birth-weight (IUGR) is not only a risk factor for ADHD but also for obesity.[32] Thus, it can be inferred that ADHD and obesity have a common genetic predisposition towards a phenotype in fetal programming.[33] In regard to growth retardation,[11] children with ADHD and its pharmacotherapy have relative prepubertal shortness, but this may not be apparent until adulthood, or not at all given the possibility of catch-up growth, delayed maturity and the resolution of underlying ADHD.[34] Indeed, it is known that height related emotional and learning differentials can disappear dependent on family circumstances and educational opportunity.[35] These observations can temper the implications of the findings in the present study of associations between ADHD and stature. Most importantly are the several co-existent morbidities, any one or several of which could have interrupted or stalled growth temporarily or permanently. To this must be added the contribution of socioeconomic factors, care and nutrition to neurodevelopment which exceeds those for linear growth, but are of functional consequence.[35,36] While short stature and associated morbidities may prompt nutritional assessment and intervention, its accelerated velocity and maximization ought not be the primary objective Whether linear growth and body fatness are interactive with the ADHD comorbidities for ADHD expression and severity is of pathogenetic relevance. Without food and nutrient intake data in this population, the question of food and nutrient therapies is mute, but the safest nutrition approach will be that of dietary pattern with attention to quality by way of biodiversity.[37,38] Specific nutrient interventions without evidence of deficiency would not satisfy a risk-benefit analysis.[39]

Drug therapy has been the first choice for children over 6 years old with ADHD. Central stimulants are used for children with ADHD. Their side effects are mainly manifest in early with loss of appetite, weight loss, reduced growth velocity and disordered sleep, which are mostly clinically manageable and tolerated.[40] However, the negative media reports and the stigma surrounding the social attitude towards the use of these drugs make it difficult for parents to accept medical treatment,[41] especially for children with ADHD who are stunted and wasted, so that parents decline the drugs and discontinue their use at an early stage.[42-44] A longitudinal community study in Australia followed up children with ADHD and found that the stimulant use was only 13.6% among children younger than ten-years-old, but increased to 25.6% thereafter.[45] Children with ADHD experience emaciation and growth retardation before the age of 10. But medication usage is planned long term when the nutritional problems of weight loss and growth retardation become increasingly problematic for clinicians and parents.[46] The European guidelines for the management of adverse drug reactions (ADRs) associated with ADHD suggest that body weight and height should be monitored every six months during drug treatment. If the growth rate is impaired, food therapy with energy dense and nutritious snacks and meals, rather than stopping the drug, is recommended. The nutrition management of children with ADHD is regulated in some jurisdictions.[47] As shown in Table 3, the risk of ADHD is increased in children with premature birth, allergic diseases, otitis media, tonsil or adenoid hypertrophy, gastroesophageal reflux, and poor sleep quality, consistent with other reports.[6,33] Not only are patients with the identified comorbidities prone to nutritional problems, but some evidence points to food or nutrient deficiency as contributory to ADHD itself.[48] Thus, dietary management is likely to need individualisation and professional clinical nutrition input with diet planning and supplementation as appropriate.[49] Recourse has been sometimes found justifiable for polyunsaturated fatty acid and vitamin D

supplements and particular attention to diets replete with zinc, iron and magnesium, apparently conducive to improvement in clinical symptomatology and overall nutritional status in children with ADHD.[50] The nutritional management of children with ADHD is demanding for clinicians, parents and child.

Limitations

Hierarchical cluster sampling for participants in this study of ADHD accessed more than 20,000 school-age children in different schools in 9 major cities in China. While the sample size was large with good overall representativeness, it does not necessarily reflect the overall distribution of ADHD and the associated nutritional problems, especially as they relate to similar if less well-defined health problems. Diagnosis ultimately depended on parental recall and communication which is inevitably imperfect Secondly, that problems were nutritional depends on the presumption that premature birth, height and body composition reflect, respectively, maternal diet and intrauterine nutrition in the first case, and the child's diet and nutritional biology in the second and third cases. Thirdly, this study is cross-sectional study so that associations may suggest, but not assure that any are those of cause and effect.

Conclusions

The nutritional status of school-age children with ADHD in China and elsewhere is complex, but most evident by an increased prevalence of short stature which may, in part, be attributable to dietary factors; and in overweight and obesity, a reflection of energy dysregulation. This is accompanied by an increased history of premature birth, immunoinflammatory and allergic disease, pharyngo-respiratory disease, gastroesophageal reflux, and poor sleep quality. Separately and together, these problems are likely to exacerbate any nutritional disorder and create a spiral of ill-health. The association with premature birth points to the critical importance of policies which address the first 1000 days or life from conception as crucial in the nutritional optimisation of human development. The prospects for better health in children at risk of and affected by ADHD may be advanced by greater regard to their nutritional biology and care.

AUTHOR DISCLOSURES

The authors declare no conflict of interest.

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Original Article

Cognitive function and elderly macronutrient intakes from rural diets in Qingdao, China

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Background and Objectives: Energy provided by macronutrients plays a key role in healthy aging. This study therefore explored the association between macronutrients and cognitive function in elderly populations in rural areas of Qingdao, China. **Methods and Study Design:** This study included 1,504 participants over the age of 65 recruited from Licha Town, Qingdao City, China. Dietary intake was measured using the Food Frequency Questionnaire, and cognitive function was assessed using the Mini-Mental State Examination. Logistic regression models were used to evaluate the association between dietary macronutrient intake and cognitive function. In addition, restricted cubic bars were applied to determine the dose–response relationship between macronutrient ratios and cognitive performance. **Results:** A total of 877 adults over the age of 65 were included. After adjusting the weighted multiple variables, significant positive associations were revealed between protein and moderate carbohydrate intake and cognitive ability, but a negative association between fat intake and cognitive performance was identified. After calculating the daily energy supply ratio, similar associations were revealed between fat and protein intake and cognitive function. Furthermore, the ratio of proteins to carbohydrates had a U-shaped relationship with cognitive function ($p_{nonlinearity}$=0.674), whereas the ratio of proteins to fats was L-shaped with lower cognitive function ($p_{nonlinearity}$<0.001). Compared with the lowest quartile of the ratio of protein to fat intake, the weighted adjusted OR (95% CI) of the highest quartile was 0.509 (0.314, 0.827) for low cognitive performance. **Conclusions:** With an adequate carbohydrate supply, appropriately increasing dietary protein intake and reducing fat intake might benefit the cognitive function of elders in rural areas.

Key Words: macronutrients, cognitive function, older adults, rural area, ratios

INTRODUCTION

Improvements in socioeconomic conditions and medical treatment have resulted in a larger aging population globally, inevitably leading to increased health problems. The aging process is one of physiological decline, including in muscle, bone, and cognitive function. Cognitive function plays a key role in the health and living standards of the elderly population.[1] Mild cognitive impairment (MCI) is a common disease in the senior population. It is characterized by a decline in memory, attention, and cognitive function and is considered a transitional stage of evolutionary dementia with a conversion rate of 10%–15% per year.[2] Alzheimer disease (AD), also a form of dementia, is becoming a global health problem as the elderly population continues to increase.[3] Census data has predicted that by 2050, 13.8 million people in the United States alone will be diagnosed with AD.[4] The progression from cognitive decline to AD is a continuous irreversible process, thus taking steps to reduce or delay the onset of MCI and dementia is crucial. Because no definitive treatment for MCI or dementia currently exists, identifying lifestyle and other risk factors affecting cognitive function is essential for the prevention and treatment of AD.[5]

Dietary patterns and the intake of nutrients are reported to be closely associated with cognitive function. Food nutrients, such as long-chain omega-3 polyunsaturated fatty acids (n3-PUFA) and polyphenols including resveratrol and flavonoids, are likely to be beneficial to cognitive function.[6-9] In addition, a study based on the National Health and Nutrition Examination Survey revealed that dietary and total zinc, copper, and selenium intake were inversely associated with the prevalence of low cognitive performance, suggesting that dietary nutri-

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tion intervention might prevent cognitive decline.[10]

Currently, the focus in nutrition is returning on energy and macronutrients. Carbohydrates, proteins, and fats are the three essential energy-yielding nutrients for physical functions, and they play a key role in many biological processes. As structural and cell surface components, they are involved in essential cell recognition processes and metabolism.[11] Carbohydrates are the main energy source, providing energy for brain activity.[12] Amino acids in proteins are precursors of key neurotransmitters and play a vital role in neuromodulation,[13] and a meta-analysis of randomized controlled trials determined that fatty acids, such as n3-PUFA, could be mildly beneficial for improving memory function in older adults without dementia.[6] By contrast, multiple studies have demonstrated that high fat intake leads to the excessive production of circulating free fatty acids and inflammation throughout the body, which adversely affects cognitive function.[14,15] A study also reported that high protein intake was significantly associated with the increased frequency of MCI.[16]

Carbohydrates, proteins, and fats interact to maintain normal cognitive function; thus, some studies have attempted to analyze their association with cognitive function by calculating the proportion of energy provided by each of them.[16,17] However, epidemiological data on the association between macronutrients and cognitive decline in regions with a relatively low economic level, such as rural areas in China, remain limited. Therefore, we conducted a cross-sectional study to explore the association between macronutrients and cognitive function in the elderly population in a rural area of Qingdao, China. In addition, we estimated the percentage of energy supply in macronutrients, calculated the ratios of pairs of macronutrients based on energy intake, and explored potential dose–response relationships.

METHODS

Research participants

The cross-sectional study was conducted from January to July 2019 in Licha Town, Qingdao City, China. In this survey, researchers underwent uniform training and recruited 1,504 participants through face-to-face interviews. The inclusion criteria were as follows: People aged over 65, lived in the area for at least a year, were capable of self-managing daily life, without self-recognized cognition dysfunction and willing to participate in the study. Exclusion criteria for the participants were as follows: Individuals with severe illnesses (e.g., cancer, severe psychiatric disorders, recent history of heart or respiratory failure, chronic renal or liver failure); individuals with conditions known to influence cognitive function (e.g., alcohol abuse, cerebral apoplexy and infraction); individuals with neurological disease (e.g., AD, Parkinson's disease (PD)) or long-term frequent intake of antidepressants and other medications for neurological diseases; individuals lack of information on cognitive function and dietary intake. The study protocol was approved by the Medical Research Ethics Committee of Qingdao Center for Disease Control and Prevention (ID SPAQ-2016-125), and all participants were provided with written informed consent during the survey period and allowed to terminate their participation any time. Ultimately, 877 participants (395 men and 482 women) were included in this study.

Assessment of dietary intake

Dietary intake was measured by 97-item (such as whole grain, red meat, pork, beef, mutton, chicken, fish, vegetables, milk, eggs, fruit, nuts, cooking oil, soybeans and their products) Food Frequency Questionnaire (FFQ) and the dietary intake data was assessed through face-to-face recall interviews. The frequency (daily, weekly, monthly, or yearly) and how much they ate were studied. Based on the Chinese Food Ingredients Table (6th edition), the nutrient intake was converted using a nutrition calculator. The residual method was used to adjust the energy of each nutrient.

Assessment of cognitive function

Cognitive function was evaluated by the Chinese version of the Mini Mental State Examination (MMSE), which possessed good validity and reliability in cognitive screening and was applicable to the preferred scale for dementia screening.[18] As a cognitive screening test, MMSE is easy to operate and widely used. The scale includes the following 7 aspects: time orientation (5 points), location orientation (5 points), immediate memory (3 points), attention and calculation ability (5 points), delayed memory (3 points), language (8 points) and visual space (1 point). In this study, time and place orientation assessment required participants to tell the year, month, day, season and day of the week on the test day as accurately as possible, and to tell their city, district or county, street or township, community or village, and several floors as far as possible. The scores for each section were expressed in exact quantities that can be answered, ranging from 0 to 5. The immediate memory assessment required participants to repeat all three items after the questioner had said their names, with scores ranging from 0 to 3 on the correct number of items they could answer. The attention and calculation ability assessments asked participants to subtract 7 from 100 and say what was the result of each calculation, with scores on a scale of 0 to 5 expressed as the correct number of answers. The delayed memory assessment asked participants to repeat three of the things you have said before, with scores ranging from 0 to 3. For language assessment, participants were asked to performed as the instructions they heard, identify the items and name them correctly, with scores ranging from 0 to 8. The visual space assessment required participants to imitate the example diagram for drawing, and the score was based on the degree of characteristic fit of the simulated picture, and the score was 1. With a total score between 0 and 30, the higher the scores suggested the better the cognitive function. As is known, the MMSE is strongly influenced by educational background and varying cut-offs stratified by educational level are recommended for the purpose of improving the effectiveness of the screening. Consequently, the assessment criteria for cognitive impairment were: ≤17 for illiteracy individuals, ≤20 for individuals with 1-6 years of education, and ≤24 for individuals with 7 or more years of education. Meanwhile, a neurologist participated in our

survey and assisted our cognitive evaluation with his clinical experience.

Covariates

We selected covariables based on the literature, MMSE scores and potential risk factors for cognitive function. This research included the self-reported questionnaire research of individuals' age, sex, educational level, income, smoking, drinking, history of chronic disease (diabetes, hypertension, dyslipidemia, heart problems and gout), Body mass index (BMI) and physical activities. BMI was calculated as the weight (kg) / height (m^2). Mini Nutritional Assessment Short Form (MNA-SF) was used to measure nutritional status, with score ranging from 0 to 14 and a higher score indicating better nutritional status.

Statistical analysis

If the continuous variables were normally distributed, the data was presented by mean and standard deviation (SD), otherwise by median and quartile range (IQR). According to the distribution of continuous variables, factorial design ANOVA analyses or Wilcoxon rank sum test were used to compare the average of continuous variables between the MCI group and the normal group. Pearson's Chi-square test or Fisher's Exact test were used to compare the distribution of category variables between the cognitively underperforming group and the cognitively normal group. Proteins, fats, carbohydrates, protein to fat ratio, protein to carbohydrate ratio, and fat to carbohydrate ratio were classified as quartiles and the higher the quartile, the higher the intake. Logistic regression analysis was used to study the relationship between macronutrients and cognitive function, and the intake of the lowest quartile was used as a reference. Rough models did not adjust any confounding factors, multivariate adjustment model for age (years), sex, marital status, smoking, drinking, chronic disease, BMI, physical activities and energy.

We used restricted cubic plot to further investigate the dose-response relationship between the ratios of macronutrient intake and low cognitive function after adjusting for the confounders. A multivariate linear regression model was established to analyze the MCI with multivariate adjusted ORs as the dependent variable and the ratios between macronutrients as the independent variable. All statistical analyses were performed using SPSS software (version 26.0) and Stata 15.0 (Stata Corporation, College Station, TX, USA). All the statistical analyses were performed at the conventional two-tailed alpha level of 0.05.

RESULTS

Dietary patterns and characteristics of the population by cognitive performance status

The dietary patterns of the elderly population in the rural area studied are presented in Supplementary Table 1. The consumption of grains, vegetables, red meat and poultry, eggs, beans, and nuts met dietary guideline recommendations, the consumption of plant oils was considerably higher than that recommended, but the consumption of whole grains, potatoes, and fruit was positively associated with cognitive function at Q2 and higher quantiles (Q3, Q4), respectively. slightly less than that recommended. The local elderly population seldom ate aquatic products.

Sample characteristics in relation to various measures of low cognitive performance are summarized in Table 1. Differences in sex, age, marital status, MNA, calf-circumference, and activity time between those with MCI and those with normal cognitive function were significant ($p<0.001$). Men ($p=0.041$), those of older age ($p<0.001$), and people who were unmarried or widowed were more likely to have poor cognitive performance. Compared with people with normal cognitive function, people with lower cognitive ability were more likely to have smaller calf and hip circumferences, higher rates of poor nutrition, and less daily activity.

Association between intake of macronutrients and the risk of MCI

Elderly people with normal cognitive performance consumed significantly more energy and macronutrients than those with MCI (Table 1). A higher percentage of fat energy supply was observed in elderly people with MCI, but this result was nonsignificant ($p=0.116$). The dietary intake of macronutrients was divided into four quartiles (Q1–Q4). Table 2 summarized the association between macronutrients and cognitive function. The OR with a 95% CI for cognitive function demonstrated that dietary protein intake was significantly positively associated with cognitive function. However, dietary fat intake was not significantly associated with cognitive function, but a higher intake of dietary carbohydrates (Q3, Q4) was significantly positively associated with cognitive function. After adjusting for potential confounders (age, sex, marriage, smoking, alcohol consumption, hypertension, diabetes, coronary heart disease, gout, dyslipidemia, daily activity time, and BMI), the same associations with cognitive function were identified for proteins, fats, and carbohydrates. After adjusting for energy, dietary protein intake was significantly positively associated with cognitive function. For dietary fat intake, the OR (95%CI) of Q2 was 1.614 (1.010–2.579), and the OR (95%CI) of Q3 was 1.823 (1.093–3.041). The OR (95%CI) of Q3 for carbohydrate intake was 0.528 (0.290–0.963).

With regard to percentages of energy supply (Supplementary Table 2), high protein energy supply ratios were positively associated with cognitive function, but the association was not linear. A high fat (Q4) energy supply ratio was negatively associated with cognitive function. No significant association between carbohydrate energy supply and cognitive function was identified.

Association between ratios of macronutrient intake and the risk of MCI

To further analyze the association between macronutrients and cognitive function, we calculated the ratio of pairs of macronutrients based on energy intake, as presented in Table 3. The OR of the 95% CI for cognitive function indicated that the highest ratio (Q4) of fats to carbohydrates exhibited a negative association with cognitive function. The ratios of proteins to carbohydrates and proteins to fats were significantly positively associated with cognitive function at Q2 and higher quantiles (Q3, Q4), respectively.

Table 1. Characteristics of the population by cognitive performance status

	Normal cognitive performance	MCI	*p*
Number	671	206	
Gender, Male, n (%)	315 (46.9)	80 (38.8)	0.041
Age (years)	71.54 (5.59)	74.78 (6.69)	<0.001
Education, n (%)			<0.001
Illiteracy	252 (37.6)	129 (62.6)	
Primary school	253 (37.7)	57 (27.7)	
Secondary school and above	166 (24.7)	20 (2.3)	
Marital status, n (%)			<0.001
Spinsterhood	4 (0.5)	4 9(1.9)	
Married	529 (78.8)	130 (63.1)	
Widowed	138 (20.6)	72 (35.0)	
Smoking, n (%)	176 (26.2)	45 (21.8)	0.205
Alcohol drinking, n (%)	184 (27.4)	45 (21.8)	0.111
MNA, n (%)			<0.001
Good nutritional status	553 (82.4)	148 (71.8)	
Underlying malnutrition	117 (17.4)	52 (25.2)	
Malnutrition	1 (0.1)	6 (2.9)	
BMI, kg/m^2	24.6 (3.84)	24.5 (3.50)	0.177
Calf circumference, cm	34.2 (3.64)	32.6(3.09)	<0.001
Waist circumference, cm	91.5 (36.8)	89.8 (9.13)	0.511
Hip circumference, cm	97.6 (8.47)	96.1 (±8.55)	0.031
Chronic disease history, n (%)			0.199
0	295 (44.4)	81 (41.1)	
1	255 (38.4)	69 (35.0)	
2	81 (12.2)	37 (18.8)	
3	20 (3.0)	5 (2.5)	
>3	13 (2.0)	5 (2.5)	
Physical activity, h/d	2.54(1.97)	2.00(1.77)	0.001
Energy intake, kcal/d	2088 (812)	1760 (769)	<0.001
Protein intake, g/d	81.0 (38.3)	62.44 (31.9)	<0.001
Animal sources, g/d	21.6 (16.6)	15.3 (11.7)	<0.001
Plant sources, g/d	59.8 (36.6)	48.3 (32.2)	0.056
Fat intake, g/d	73.3 (40.6)	65.3 (38.1)	0.002
Animal sources, g/d	21.8 (20.9)	14.35 (14.6)	<0.001
Plant sources, g/d	51.6 (30.4)	46.8 (26.8)	0.002
Carbohydrate intake, g/d	277 (129)	231 (127)	<0.001
Fiber intake, g/d	20.3 (9.75)	16.2 (9.34)	<0.001
Protein/energy, %	15.5 (3.9)	14.3 (4.4)	<0.001
Fat/energy, %	31.9 (12.5)	34.4 (14.9)	0.006
Carbohydrate/energy, %	52.7 (11.8)	51.4 (13.9)	0.116

After adjusting for potential confounding factors (age, sex, marriage, smoking, alcohol consumption, hypertension, diabetes, coronary heart disease, gout, dyslipidemia, daily activity time, and BMI), similar results were observed. After adjusting for energy, the ratio of proteins to fats was positively associated with cognitive function, and the ratio of proteins to carbohydrates exhibited a positive association with cognitive function at Q2 and Q3 quantiles. No significant association was identified in the fats to carbohydrates ratio.

To further and clearly reflect the dose–response relationships, a restricted cubic spline analysis was used, as depicted in Figure 1. The relationship between the ratio of fat to carbohydrate energy intake and cognitive function was not significant. For low cognitive function, we identified an L-shaped association with the ratio of proteins to fats and a U-shaped association with the ratio of proteins to carbohydrates. The prevalence of low cognitive function decreased with an increase in the protein to fat energy intake ratio in a linear dose-dependent manner ($p_{nonlinearity}$<0.001), and with an increase in the protein to carbohydrates ratio, a nonlinear dose-dependent relationship ($p_{nonlinearity}$=0.674) was observed. The MCI OR values exhibited a decreasing and then increasing trend.

DISCUSSION

Summary of the main findings

In this population-based cross-sectional study of older adults, we analyzed the association between three macronutrients (carbohydrates, proteins, and fats) and cognitive function. After adjusting for influencing confounders, these macronutrients were significantly associated with cognitive ability. Moreover, the ratio of proteins to fats, based on energy intake, exhibited a significant dose–response association with the cognitive performance of the elderly population.

Energy is essential for living things to maintain their basic functions. The total amount of macronutrients and the proportion of energy supply are closely related to a variety of physical functions, such as cardiovascular function, cognitive function, exercise ability, and basic metabolism.[19,20] As one of the three productive nutrients,

Table 2. Population odds ratios (95% confidence intervals) of cognitive performance status by quartiles of macronutrient intakes

Variables (g/d)	Normal	MCI	Model†	Model‡	Model§
Protein					
Q1 (lowest-52.4)	135	85	1 (reference)	1 (reference)	1 (reference)
Q2 (52.4-72.3)	174	45	0.411 (0.268, 0.629)	0.406 (0.259, 0.635)	0.437 (0.266, 0.719)
Q3 (72.3-97.7)	175	45	0.408 (0.267, 0.625)	0.430 (0.275, 0.670)	0.485 (0.275, 0.856)
Q4 (97.7-highest)	187	31	0.263 (0.165, 0.420)	0.285 (0.174, 0.465)	0.354 (0.160, 0.784)
Fat					
Q1 (lowest-42.2)	164	56	1 (reference)	1 (reference)	1 (reference)
Q2 (42.2-62.0)	161	59	1.073 (0.701, 1.642)	1.165 (0.747, 1.817)	1.614 (1.010, 2.579)
Q3 (62.0-95.0)	167	51	0.894 (0.578, 1.384)	1.043 (0.659, 1.651)	1.823 (1.093, 3.041)
Q4 (95.0-highest)	179	40	0.654 (0.414, 1.034)	0.786 (0.486, 1.273)	1.799 (0.995, 3.251)
Carbohydrate					
Q1 (lowest-173)	144	75	1 (reference)	1 (reference)	1 (reference)
Q2 (173-252)	161	59	0.704 (0.468, 1.059)	0.723 (0.472, 1.108)	0.868 (0.538, 1.402)
Q3 (252-339)	183	36	0.378 (0.240, 0.594)	0.386 (0.241, 0.617)	0.528 (0.290, 0.963)
Q4 (339-highest)	183	36	0.378 (0.240, 0.594)	0.401 (0.249, 0.646)	0.695 (0.310, 1.561)

†Crude model did not adjust any confounders.
‡Adjusted for age (years), gender, marital status, smoking status, alcohol drinking, chronic diseases, BMI and the activity time.
§Adjusted for age (years), gender, marital status, smoking status, alcohol drinking, chronic diseases, BMI, the activity time and the total energy.

Table 3. Population odds ratios (95% confidence intervals) of cognitive performance status by quartiles of macronutrient intakes' ratio

Variables (g/d)	Normal	MCI	Model†	Model‡	Model§
Protein					
Q1 (lowest-0.24)	151	68	1 (reference)	1 (reference)	1 (reference)
Q2 (0.24-0.29)	182	37	0.451 (0.286, 0.711)	0.436 (0.271, 0.701)	0.403 (0.249, 0.653)
Q3 (0.29-0.37)	169	50	0.657 (0.429, 1.006)	0.647 (0.414, 1.010)	0.605 (0.384, 0.952)
Q4 (0.37-highest)	169	51	0.670 (0.438, 1.024)	0.764 (0.490, 1.190)	0.690 (0.439, 1.085)
Fat					
Q1 (lowest-0.36)	178	43	1 (reference)	1 (reference)	1 (reference)
Q2 (0.36-0.56)	169	47	1.151 (0.724, 1.831)	1.167 (0.721, 1.887)	1.045 (0.641, 1.703)
Q3 (0.56-0.93)	167	53	1.314 (0.834, 2.069)	1.351 (0.843, 2.166)	1.256 (0.777, 2.030)
Q4 (0.93-highest)	157	63	1.661 (1.066, 2.587)	1.755 (1.108, 2.780)	1.542 (0.964, 2.466)
Carbohydrate					
Q1 (lowest-0.35)	151	68	1 (reference)	1 (reference)	1 (reference)
Q2 (0.35-0.52)	168	52	0.687 (0.450, 1.049)	0.657 (0.420, 1.027)	0.620 (0.393, 0.978)
Q3 (0.52-0.76)	171	48	0.623 (0.406, 0.958)	0.592 (0.377, 0.929)	0.566 (0.358, 0.896)
Q4 (0.76-highest)	181	38	0.466 (0.297, 0.733)	0.457 (0.285, 0.734)	0.496 (0.307, 0.801)

†Crude model did not adjust any confounders.
‡Adjusted for age (years), gender, marital status, smoking status, alcohol drinking, chronic diseases, BMI and the activity time.
§Adjusted for age (years), gender, marital status, smoking status, alcohol drinking, chronic diseases, BMI, the activity time and the total energy.

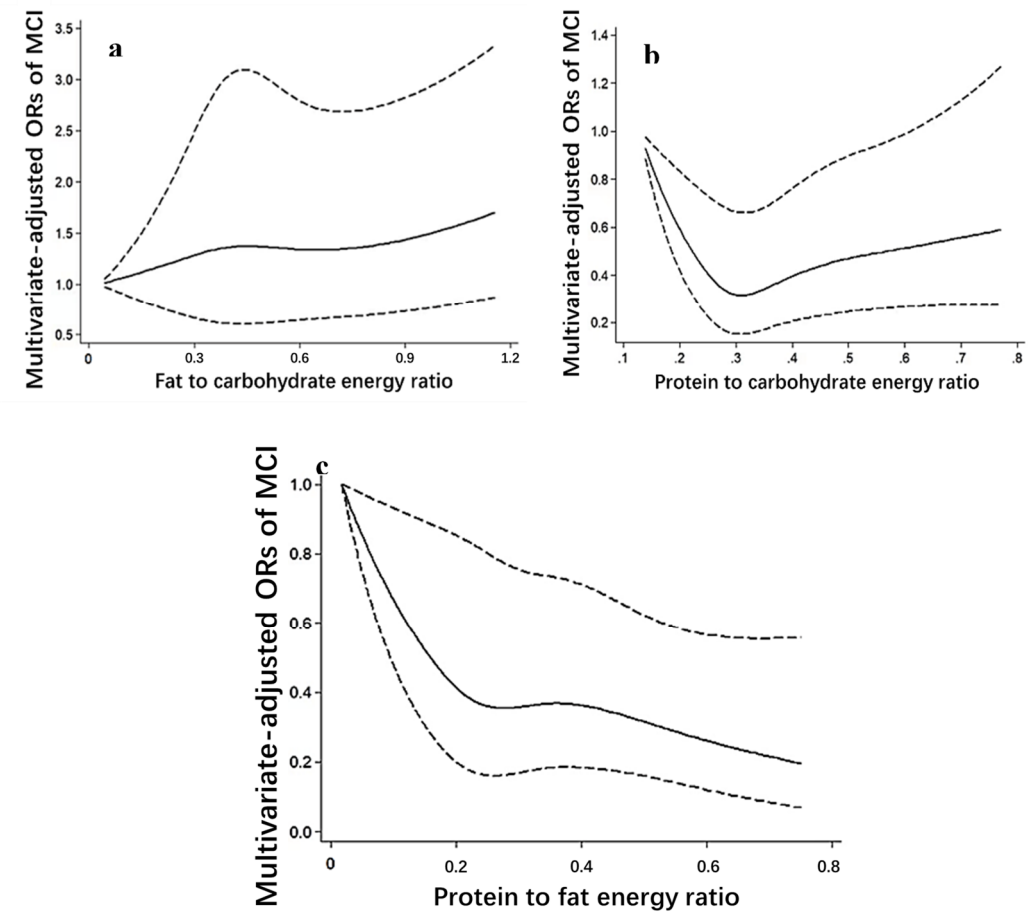

Figure 1. Restricted cubic spline model of the ORs of low cognitive performance (MCI) with ratios of nutrient intake for productivity, adjusted for age (years), gender (male, or female), BMI, smoking status, alcohol drinking, occupation, marriage, total daily energy intake (kcal/d). The solid lines represent the ORs, and dashed lines represent the 95% CIs. b) $p_{\text{nonlinearity}} = 0.674$; c) $p_{\text{nonlinearity}} < 0.001$.

proteins, such as those in lean meat and milk, play a key role in the maintenance of the human body's functions and health, and numerous studies have reported an association between dietary protein intake and cognitive function. Data on the US population aged 60 and older demonstrated positive associations between dietary protein intake and performance in a variety of cognitive domains. In addition, Roberts et al observed a significant association between dietary protein intake amounting to 16% to 20% of total energy intake and a reduced risk of MCI or dementia.[21] Our study also demonstrated that higher dietary protein intake was associated with improved cognitive function.[22] Tryptophan and tyrosine are precursors of key neurotransmitters that regulate cognitive function by influencing the synthesis and release of serotonin (5-hydroxytryptophan) and catecholamine neurotransmitters (dopamine, norepinephrine, and epinephrine).[23] Animal and human studies have demonstrated that tryptophan deficiency reduces signaling to serotonin receptors and tyrosine weakens dopamine function, leading to impairment in some areas of cognition.[24,25] Providing tryptophan and tyrosine supplements might have beneficial effects on emotional functioning and cognitive tasks in older adults.[26] In addition, several amino acids, such as arginine (nitric oxide and polyamines), histidine (histamine), and serine (phosphatidylserine), act as neuromodulators that affect cognitive function.[27] Moreover, malnutrition and weakness could lead to cognitive decline, and proteins could improve and prevent these conditions.[28] Therefore, we hypothesized that dietary proteins might prevent cognitive decline by improving nutritional status and vulnerability.[29] Studies have also demonstrated that the overconsumption of protein might be harmful to cognition, which is not consistent with our results.[16] However, our results demonstrated that Q3 of the protein energy supply ratio performed the best cognitive function, indicating that not the higher the protein intake is, the better cognitive performance is. The elderly population in the rural areas surveyed was still largely reliant on the crops they grew for their diet, and with their proteins derived primarily from grains, they required a higher protein intake to maintain cognitive function. But that did not mean that more protein was better. Overconsumption should also be avoided.

Fats are another major source of energy for the body and produce heat to protect the body from the cold. Many studies have verified the relationship between dietary fat intake and total intake with cognition function. Prospective population studies have demonstrated that the intake of total fat, high saturated fat, and cholesterol increase the risk of dementia. Total fat is particularly associated with vascular dementia because of its impact on cardiovascular

structure and function, and thus, it is associated with AD. A randomized controlled clinical trial revealed that attention, speed, and mood were impaired in a group of young men (age: 22±1 years) who received a high-fat, low-carbohydrate diet for five days, suggesting that a high-fat diet might be harmful to the brains of healthy individuals.[30] In animal studies, rats on a long-term high-fat diet developed hippocampal microvascular insulin resistance and significantly reduced cognitive function.[31] As human and animal studies have demonstrated, this mechanism might be responsible for the increased risk of MCI caused by high fat intake. These studies are consistent with our findings that a high-fat diet is significantly negatively associated with cognitive function; the intake of unsaturated fat is also reported to be negatively associated with such function. Eating fish or foods containing polyunsaturated fatty acids could reduce this risk, such as in the "Mediterranean diet." N-3 fatty acids are known to be beneficial for neuroprotection,[32] but the intake of these fatty acids by the rural elderly population we surveyed was relatively low because of the low intake of aquatic products. We therefore hypothesized that the overconsumption of oils might be responsible for the increased risk of MCI.[33]

Carbohydrates are the largest productive nutrient and play a key role in the body's energy supply, making them essential for the prevention and treatment of cognitive diseases -they provide the nervous system with glucose, which is required continuously for normal function. A study of nutritional strategies for optimizing the cognitive function of the aging brain noted that adequate carbohydrate intake is critical to brain function because glucose is the main energy source for the brain. The brain requires about 25% of the total glucose energy consumed despite only comprising 2% of total body weight.[34] By contrast, a chronic excess of glucose consumption could lead to reduced synaptic plasticity and high levels of inflammation, which might contribute to cognitive deficits.[35] Notably, cognitive fatigue is common in older adults, which might be due to a decline in neuronal glucose utilization with age, possibly as the result of reduced brain sensitivity to insulin.[36]

Although all three macronutrients are associated with cognitive function, their proportions might be of more importance to cognitive function with respect to meeting energy requirements. Ding et al revealed a high percentage of energy intake from fats and proteins with low-energy intake from carbohydrates might be associated with cognitive decline.[16] We also analyzed the association between the energy supply ratio of macronutrients and cognitive function. Because the effect of the relationship among the three nutrients on cognition is not well understood, we calculated the ratio of pairs of productive nutrients based on energy intake and evaluated the dose–response relationship between cognitive function and these ratios. We determined that the energy ratio of fats to carbohydrates had no significant effect on cognitive function. This result indicates that neither high-carbohydrate and low-fat nor high-fat and low-carbohydrate diets are strongly associated with cognitive function. However, the energy ratios of proteins to carbohydrates and proteins to fats were revealed to have a significant effect on cognitive function, indicating that proteins might play a key role in cognitive function in the elderly population. As for the ratio of proteins to carbohydrates, we observed that the OR value decreased gradually with the increase in the ratio of proteins to carbohydrates within a certain range. When the ratio continued to increase, the OR value began to rise. We speculated that though proteins are beneficial for cognitive function, a high level of protein with a low-carbohydrate diet was not superior to the ratio at a moderate level. Notably, the energy ratio of proteins to fats exhibited a linear dose–response relationship with cognitive performance, suggesting that a higher amount of proteins relative to fats is more conducive to the maintenance of cognitive function with the same energy supply. As discussed previously, the fat intake of the elderly population in rural areas is mainly saturated fatty acids and n-6 polyunsaturated fatty acids (vegetable oil), and the intake of n-3 PUFA is low. However, the elderly population in rural areas might consume a certain amount of plant protein. The phytochemicals in plant-based food might help protect cognitive function; hence, a higher energy ratio of proteins to fats would benefit cognitive function. When this ratio increases, however, the OR value for MCI flattens, suggesting that too high a protein to fat ratio might not result in improved cognitive performance. In addition, the ratio of proteins to carbohydrates indicates that a diet too low in carbohydrates would also not improve cognitive performance; thus, the carbohydrate energy supply is essential for maintaining cognitive function. This is somewhat consistent with the macronutrient composition of healthy dietary patterns we reported previously, which was based on the consumption of rice and flour, red meat, poultry, vegetables, aquatic products, and fruits, which protect against cognitive dysfunction.[37] Therefore, we speculated that to maintain a sufficient carbohydrate energy supply, increasing the ratio of proteins to fats might be beneficial to the maintenance of cognitive function in the elderly population in rural areas.

In addition to the intake of macronutrients, we identified other potential risk factors for MCI in this study. Because of differences in socioeconomic status, physiological status, and access to health services, women have a significant cognitive disadvantage over men.[38] Moreover, studies have revealed that aerobic exercise could increase the volume of the hippocampus in later life and have a positive effect on memory performance, which is consistent with our findings.[39]

Advantages and limitations

Our study has several advantages. A major advantage is that we evaluated the association between macronutrients and cognitive function in a rural elderly population and also explored the association between their macronutrient ratios and cognitive function. The inverse relationship between "ratio of protein to fat intake" and low cognitive performance remained significant after adjustments for major confounders. In addition, we used restricted cube splines to investigate the dose–response relationship between each ratio and cognitive performance. Finally, combined with the results of our previous studies, we determined that lower consumption of coarse cereals, potatoes, fruits, red meat and poultry, eggs, and nuts was

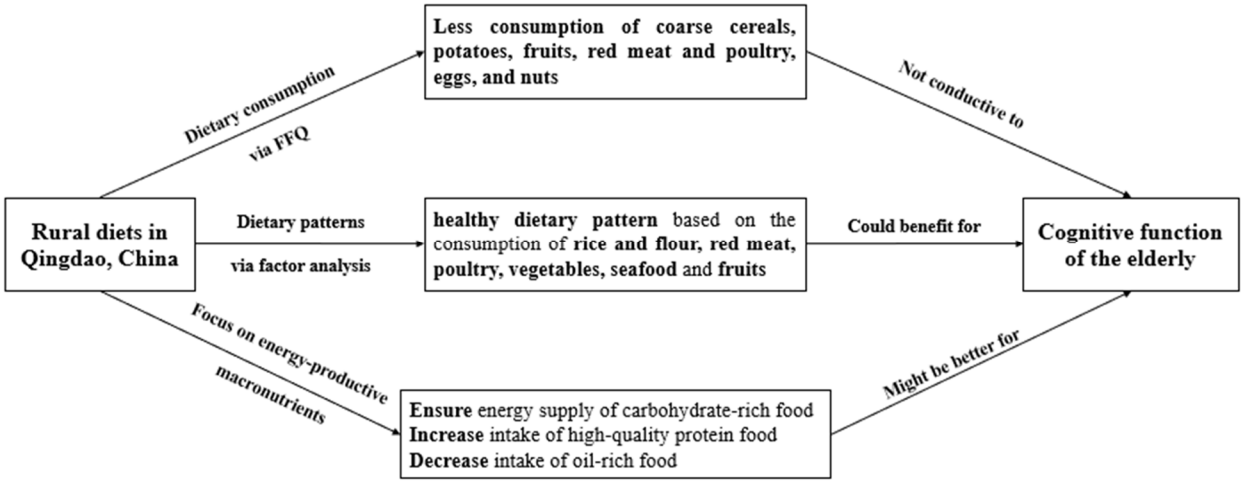

Figure 2. The association between rural diets and cognitive function in the elderly in Qingdao, combining with our previous work.

not conducive to cognitive function in the elderly population in rural areas in Qingdao. We also analyzed dietary patterns through a factor analysis and determined that healthy dietary patterns based on the consumption of rice and flour, red meat, poultry, vegetables, aquatic products, and fruits could benefit cognitive performance. In this study, we focused on energy-productive macronutrients and further analyzed the macronutrient intake characteristics of the diet of elderly people in rural areas, providing appropriate recommendations; we have clarified the association between diet and the cognitive function of the elderly population in the rural areas of Qingdao (Figure 2).

The potential limitations of our research should be acknowledged. First, this was a cross-sectional study, and the risk of unmeasured confounders from a large number of dietary, environmental, and lifestyle factors was high; we were thus unable to determine a causal relationship between dietary intake and poor cognitive performance. Further prospective cohort studies are necessary to confirm temporal relationships because individuals with the lowest cognitive performance might make poor decisions about their metabolic or activity status, and therefore, they might have lower levels of nutrient intake. Second, our dietary data were collected using the FFQ. Although this instrument is considered to have high validity, recall bias might exist in the self-reported dietary intake. Third, hypertension and diabetes were self-reported, which may lead to information bias. Moreover, this study only analyzed and discussed the energy supply ratio between macronutrients without further distinguishing the sources of the nutrients. To better understand the underlying mechanisms, macronutrients from different food sources should be analyzed separately. Finally, because the participants were recruited only from the rural area of Qingdao, any generalizations from the results of this study to other places and ethnic groups should be treated with caution.

Conclusions

Our study indicated that a dietary pattern with a high ratio of proteins to fats was negatively associated with the risk of MCI in older adults, and an L-type dose–response relationship was detected. Moreover, the relationship between moderate carbohydrate intake and the maintenance of cognitive function should not be disregarded. This result suggests that elderly people should increase their intake of high-quality protein-rich foods and decrease their intake of oil-rich foods by enriching their food sources and dietary structure while also ensuring an energy supply of carbohydrate-rich food such as grains, which might be of significance for the maintenance of cognitive function. However, because this was a cross-sectional study, further prospective cohort studies and research into the mechanisms involved are required to verify these findings. These findings could be valuable for guiding the elderly population in selecting an appropriate diet with balanced nutrition and benefits for their cognitive function.

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AUTHOR DISCLOSURES

The authors declare no conflict of interest.

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Supplementary table 1. Dietary patterns of the elderly in rural area of Qingdao

Food groups, g/d	Local senior citizens	Dietary Guidelines for Chinese residents (2016)
Grains and potatoes	357 (150, 664)	250-400
Whole grains and legumes	42.9 (0, 157)	50-150
potatoes	14.3 (0, 57.1)	50-100
vegetables	446 (297)	300-500
fruits	140 (0, 200)	200-350
Red meat and poultry	46.4 (8.57, 95.7)	40-75
aquatic products	1.61 (0, 15.0)	40-75
eggs	42.0 (14.3, 50.0)	40-50
Milk and milk products	17.0 (0, 104)	300
Beans and nuts	26.5 (0, 43.9)	25-35
oils	65.5 (38.5)	25-30

Supplementary table 2. Population odds ratios (95% confidence intervals) of cognitive performance status by quartiles of macronutrients energy supply ratios

Variables (g/d)	Normal	MCI	Model†	Model‡
Protein				
Q1 (lowest-12.5)	135	74	1 (reference)	1 (reference)
Q2 (12.5-14.9)	168	56	0.610 (0.427, 0.873)	0.581 (0.402, 0.839)
Q3 (14.9-17.4)	184	36	0.363 (0.245, 0.538)	0.357 (0.239, 0.533)
Q4 (17.4-highest)	175	45	0.475 (0.327, 0.690)	0.475 (0.324, 0.697)
Fat				
Q1 (lowest-22.9)	172	43	1 (reference)	1 (reference)
Q2 (22.9-31.1)	166	53	1.273 (0.860, 1.885)	1.227 (0.822, 1.831)
Q3 (31.1-41.1)	165	54	1.321 (0.894, 1.952)	1.312 (0.880, 1.956)
Q4 (41.1-highest)	158	61	1.547 (1.053, 2.271)	1.549 (1.045, 2.298)
Carbohydrate				
Q1 (lowest-44.3)	157	60	1 (reference)	1 (reference)
Q2 (44.3-53.2)	170	47	0.731 (0.502, 1.067)	0.703 (0.478, 1.036)
Q3 (53.2-61.5)	170	49	0.766 (0.527, 1.113)	0.770 (0.525, 1.130)
Q4 (61.5- highest)	165	53	0.849 (0.587, 1.228)	0.823 (0.563, 1.202)

†Crude model did not adjust any confounders.
‡Adjusted for age (years), gender, marital status, smoking status, alcohol drinking, chronic diseases, BMI and the activity time.

Original Article

Prevalence and risk for malnutrition in older Thai people: A systematic review and meta-analysis

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Background and Objectives: Malnutrition is potentially preventable in older people, but with varied reported prevalence. We assessed its prevalence, assessment methods, and risk factors in older Thai people. **Methods and Study Design:** Studies published from January 1, 2000, to September 30, 2020 were searched in Medline, EMBASE, Google Scholar, and local databases. A random-effects model was used to calculate pooled prevalence with subgroups analysis (setting of the patient, region). Forest plots displayed sensitivity and specificity for all nutritional screening tools validated against Mini Nutritional Assessment (MNA) with tests for heterogeneity. Publication bias was tested by funnel plot and Egger's test. **Results:** 71 studies (total 23,788 subjects) were included where mean age was 65.5 to 78.3 years. The pooled prevalences of malnutrition were 10.4%, 6.1%, and 5.7% by body mass index (BMI), MNA, and MNA-Short Form (MNA-SF), respectively. At-risk of malnutrition prevalence was 42.6% using the MNA and 37.8% using the MNA-SF. The pooled prevalence of malnutrition by BMI <18.5 kg/m^2 was 10.4% (95% CI 8.7-12.4). The pooled prevalence of malnutrition based on MNA was 6.1% (95% CI 3.8-9.4). It was highest among hospitalized patients and lowest in community-dwelling elders by both measures. Factors associated with malnutrition were female sex, advanced age, low education, living alone, living in rural areas, comorbidities, eating problems, and geriatric conditions. **Conclusions:** The pooled prevalence of elder malnutrition was 6-10%, depending on assessment method and study setting. Hospitalized older people were at increased risk of malnutrition. It might be ameliorated through community directed food systems.

Key Words: malnutrition, prevalence, Thai, older people

INTRODUCTION

The number of older people worldwide is increasing. The World Health Organization estimates that by 2025 the number of people aged 60 years and above will reach 1.2 billion and approximately 840 million will live in low-income countries.[1] Many comorbidities and conditions that lead to loss of physical and mental capacity are more common among older people, increasing care support needs. The overarching public health goal is to promote a healthy aging society with less dependency.

Nutrition is a key consideration for health. Malnutrition in older adults impairs the immune system,[2] increases disease severity and complication rates, increases the likelihood of falls, reduces physical function, reduces quality of life, prolongs hospital stays, increases the costs of health care, and increase mortality.[3-7] Risk factors of malnutrition in older people encompass 1) physiological factors including age, anorexia in aging, frailty, polypharmacy, chronic illness, alcohol use, decreased physical function, poor oral health, swallowing problems, dementia, Parkinson's disease, and the inability to do shopping or meal preparation and feed themselves; 2) psychological factors including loneliness, depression, and anxiety; 3) socioeconomic factors such as the location of residence, poverty, and education level.[8] However, malnutrition is both preventable and correctable, and its severity may be decreased if treated promptly.

Nutritional screening is a procedure used to find those who are malnourished or at risk of malnutrition to determine if a detailed nutritional assessment is indicated. Therefore, the evaluating tool should be easy to use and appropriate for implementation by all healthcare professionals.[9] According to a 2014 systematic review,[10] at least 33 different evaluating tools are available. The most common tools currently used in older people include, 1)

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Mini Nutritional Assessment short-form (MNA-SF),[11] a validated screening tool for all settings; 2) Short Nutritional Assessment Questionnaire (SNAQ),[12] an easy screening tool to predict weight loss at six months in community-dwelling and long-term care residents; 3) The Nutritional Risk Screening (NRS-2002)[13] a recommended tool for hospitalized patient; 4) Malnutrition Universal Screening Tool (MUST),[14] which is recommended by ESPEN to be used at a community level;[15] 5) Malnutrition Screening Tool (MST) which is comprised of two questions addressing recent unintentional weight loss and poor appetite, and was validated in acute hospital and ambulatory care.[16] Although many screening tools are available, there is no universally-accepted single objective measure or gold standard to determine nutritional status.[17]

Following screening, a nutritional assessment can determine the presence of specific problems. Commonly used tools include the Mini Nutritional Assessment (MNA)[18] which can be used in hospital, community and long-term care settings, and the Subjective Global Assessment (SGA)[19] which is used to confirm malnutrition. Other methods to ascertain malnutrition status include assessment of dietary and medication intake, anthropometric measurement such as body mass index (BMI), subcutaneous fat thickness and body composition measurement, and biochemical markers such as serum albumin, cholesterol, white blood cell count, or anemia. Body composition assessment is sometimes used to confirm the altered body composition from malnutrition. Then, the treatable causes of weight loss should be sought and managed.

The prevalence of malnutrition in older people varies in different countries.[20] One reason for this is that previously there was no gold standard definition of malnutrition, although the common international consensus is that malnutrition is an inadequate nutritional status associated with adverse clinical outcomes. The global leadership initiative on malnutrition (GLIM) criteria were proposed in 2019 as such diagnostic criteria.[21] A two-step model for risk screening and diagnosis of malnutrition was proposed. The first step is to screen for "at-risk" status using any validated screening tool and the second step is the assessment using phenotypic and etiologic criteria including unintentional weight loss, low BMI, and decreased muscle mass. However, one of the phenotypic criteria in the GLIM-2019 is decreased muscle mass, which might hinder an epidemiologic study in many settings where the access to the measurement of muscle mass is limited. Other reasons for the varied prevalence were different screening tools used and different population characteristics. Some studies have used a BMI cut-off to define malnutrition.[22] In Thailand, the reported prevalence of malnutrition among Thai older people has ranged from 0 to 84.7%.[23-93] However, the screening and assessment methods used in these studies were different and this may have contributed to the wide variation in reported prevalence. Therefore, we conducted a systematic review and meta-analysis of malnutrition status in older Thai people.

Objectives

The primary objective was to assess the prevalence of malnutrition in Thai people aged 60 years and older. The secondary objectives were to evaluate methods for screening and assessing malnutrition and to study the risk factors of malnutrition.

METHODS

The protocol followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flow diagram.[94] To avoid bias in the selection process, the search strategy and selection criteria were defined a priori. Studies that conformed to the following criteria were investigated.

Search strategy

A systematic search was performed by MC and the articles identified through electronic searches of the international databases (Medline, EMBASE, and Google Scholar) and local databases (Thai Index Medicus, Thai Medical Index, Thai LIS, and Thai Journal Citation Index). The time frame for the search was from January 1, 2000, to September 30, 2020. The language was not restricted in the search strategy. Gray literature (e.g., thesis and conference papers) was also included. The search strategy combined the terms "malnutrition" OR "malnourished" OR "nutritional status" OR "undernutrition" OR "nutrition disorders" OR "nutrition surveys" OR "nutrition assessment" AND "elderly" OR "old" OR "older" OR "aged" OR "aging" (both in English and Thai language). In addition, a manual search was performed for potentially relevant studies using existing references cited in selected articles.

Inclusion criteria and exclusion criteria

Studies were included if they reported the prevalence of malnutrition in older Thai people (≥60 years) with a minimum sample size of 30, and measured malnutrition by using standardized and validated measuring tools (questionnaire, anthropometric, or biological indices). Studies were excluded if they included patients with terminal disease or had used duplicated data.

Selection of studies

Initial search results were saved in the EndNote program. After deleting duplicate studies, two researchers (MC and CW) independently selected research papers using titles and abstracts based on the inclusion and exclusion criteria. Then, MC and CW separately reviewed the individual articles to make a final determination whether to include or discard the study. Any disagreement was discussed between the two researchers, and disagreements were resolved by WM.

Data extraction and management

MC and CW independently extracted data using a data extraction form. The form included authors, title, year of study, study design, place, setting, population characteristics, sample size, assessment methods, prevalence, and associated factors (Supplementary table 1).

Assessment of the quality of the published studies

The quality assessment and risk of bias of the included studies were assessed by MC and CW and any disagree-

Table 1. Selected studies on factors associated with malnutrition or at-risk of malnutrition in older Thai people

Author, year	Population	Method	Factors
Arsasoi K, 2013[23]	CD	BMI MNA	Less than 20 teeth OR=2.08 (95% CI=1.06-4.09) (p=0.032) Female OR=2.04 (95% CI=1.06-3.91) (p=0.032) Age of 70 years or more OR=2.75 (95% CI=1.43-5.28) (p=0.002)
Boontanon N, 2017[27]	CD	BMI MNA-SF	Age (p<0.001) Rural area (p=0.001)
Chalermsri C, 2018[29]	OP	MNA	IADL dependence OR 5.3 (95% CI=3.1-9.2) BADL dependence OR 3.6 (95% CI=2.2-6.0) Dementia OR 3.9 (95% CI=2.4-6.3) Depression OR 3.4 (95% CI=2.0-5.7) Education level (<4 years) OR 2.2 (95% CI=1.4-3.4) Cerebrovascular disease OR 2.2 (95% CI=1.2-4.1) Medication (>5 items) OR 1.9 (95% CI=1.1-3.1) Female OR 1.8 (95% CI=1.2-2.9) CCI, (each score) OR 1.4 (95% CI=1.2-1.7) Age, (years) OR 1.1 (95% CI=1.0-1.1)
Chinuntuya P, 2016[32]	IP	MST Albumin	Risk of malnutrition Length of hospital stay rs =0.46 (p<0.01) Malnutrition Length of hospital stay rs=0.35 (p<0.01)
Churak P, 2018[33]	CD	BMI	Age ≥70 years OR=5.5 (95% CI=2.3-13.0) Single OR=12.9 (95% CI=2.4-69.5) Widow/Divorce/Separation OR=3.5 (95% CI=1.5-8.2) Teeth or gum diseases OR=8.0 (95% CI=2.2-28.9) Appetite disorder OR=3.0 (95% CI=1.4-6.5)
Gaewkhiew P, 2019[36]	CD	BMI	Functional dentition PR: 0.39 (95% CI=0.16-0.95)
Kanin M, 2020[40]	OP	BMI MNA	Exercise β 0.241 (p<0.001) ADL β 0.232 (p<0.001) Underlying disease β -0.162 (p=0.004) Age β -0.140 (p=0.015) Have related people in family β 0.114 (p=0.036)
Khanrugsa S, 2017[41]	IP	NRS 2002	Depression OR 1.191 (95%CI=1.059-1.034) Length of stay OR 1.166 (95%CI=1.019-1.334) Age OR 1.170 (95%CI=1.081-1.260)
Limpawattana P, 2020[46]	OP	BMI	Depression OR 13.2 (95% CI=2.37-73.66) (p=0.003)
Samnieng P, 2011[67]	CD	MNA	Number of teeth present, number of decayed teeth, number of FTUs, chewing ability test score (p<0.05)
Srisilapanan P, 2002[76]	CD	BMI	19 or less natural teeth OR 2.42 (95% CI=1.64-3.60) (p<0.001) no natural teeth OR 2.84 (95% CI=1.67-4.85) (p<0.001)
Sriwichian T, 2016[78]	CD	BMI MNA MNA-SF	Depression Coefficients SE (b, beta) -0.22 and -0.37 p<0.001 ADL (b, beta) 0.28 and 0.32 (p=0.01)
Tubtimtong P, 2019[88]	CD	BMI MNA-SF	Female (p<0.0001) Lower education (p=0.03) Living with family (p=0.02) Comorbidities (p=0.037) Mouth dryness (p=0.002) Avoidance of eating vegetables and fruits (p=0.032) Periodontal pockets (p=0.03) Number of natural teeth (≥20 and < 20 teeth) (p=0.003) Number of functional units (≥10 FUs and <10 FUs) (p=0.029) Type of functional units (natural maxillary teeth occluding with natural mandibular teeth) (p=0.008)
Wanaratna K, 2019[89]	CD	MNA-SF	Frailty OR 2.50 (95% CI=1.23-5.09)

CD: community dwelling; OP: out-patient; IP: in-patient; BMI: Body mass index; MNA: Mini nutritional assessment; MNA-SF: Mini nutritional assessment short form; MST: Malnutrition screening tool; ADL: activity of daily living; IADL: instrumental ADL; BADL: basic ADL; CCI: Charlson comorbidities index; OR: Odd ratio; PR: Poisson regression; β: Standardized regression coefficient beta.

ment was discussed with WM. The researchers used a 10-item rating tool developed by Hoy et al.[95] The result of the risk of bias and quality assessment is presented in Supplementary table 1.

Statistical analysis for the meta-analysis

RStudio version 1.4.1193 software was used to calculate a pooled estimate of prevalence and subgroups analysis (setting of the patient, region). Forest plots were used to

display sensitivity and specificity for all nutritional screening tools validated against MNA.

We assessed heterogeneity among studies using forest plot, I-square (I2) test, and Cochran Q test. Four intervals of I^2 were used: less than or equal to 25% represents insignificant heterogeneity; 26-50% represents low heterogeneity; 51-75% represents moderate heterogeneity; and more than 75% represents high heterogeneity. We used a random-effects model to conduct the meta-analyses.

Finally, we checked for the presence of publication bias by funnel plot and Egger's test using RStudio software.

RESULTS

Study selection

The search yielded 5,086 citations, including 1,060 duplicates. Four articles were identified from checking the reference list of relevant articles and review articles for malnutrition in the Thai older people. After title, abstract, and full-text screening, 3,959 articles were excluded, resulting in 71 relevant articles that were included in this systematic review (Supplementary figure 1).

Study characteristics

Data from 71 studies were included and analyzed to obtain the pooled prevalence of malnutrition. Sixty-seven were cross-sectional studies, and four were prospective cohort studies. Fifty-one studies were conducted in a community setting, nine in hospitalized settings (cancer patients in three studies), 10 in an outpatient setting (four of which were geriatric clinics), and a single study in a nursing home for older adults. Eighteen studies were conducted in the Bangkok metropolitan region, five from the central region of the country, 20 from the northeast, 18 from northern region, three from the southern region, four from the western region, and three were national. In total, 23,788 subjects were included with the least sample size of 30 and the highest of 4753 (Supplementary table 1).

There was a low risk of bias for 32 studies, moderate risk of bias for 29 studies, high risk of bias for 9 studies, and one study could not be evaluated due to incomplete data.

The most often used method of malnutrition assessment was anthropometric measurement. Fifty-three studies used BMI, 51 of which used a cut-off value below 18.5 kg/m^2, and two studies used a cut-off below 20 kg/m^2 to indicate malnutrition. Other studies measured skinfold thickness (two studies), mid-arm circumference (two studies), and calf circumference (one study). The MNA was used in 19 studies, MNA-SF in three studies, the SGA in nine studies, and the Nutrition Alert Form (NAF) in one study. In addition, biochemical data were used to assess malnutrition, including serum albumin (six studies), total lymphocyte count (one study), hemoglobin (three studies), hematocrit (three studies), and cholesterol (one study). For nutrition risk screening, 11 studies used MNA-SF, 22 studies used the MNA, two studies used the MST, two studies used the Nutrition Risk Classification (NRC), and three studies used the Nutritional Risk Screening (NRS).

Prevalence of malnutrition by BMI

The pooled prevalence of malnutrition by BMI <18.5 kg/m^2 was 10.4% (95% CI 8.7-12.4) (Figure 1). The highest prevalence was among hospitalized patients (16.3%, 95% CI 8-30). The lowest prevalence was estimated in community-dwelling elders (9.8%, 95% CI 7.9-11.9). (Figure 1) Regionally, the highest prevalence was 14.9% (95% CI 8.5-25.0) in the north and the lowest prevalence was 6.1% (95% CI 4.5-8.2) in the Bangkok metropolitan region (Supplement figure 2). There was high heterogeneity among studies (I^2=93%, p<0.01). We performed sensitivity analysis by excluding studies with a high risk of bias (four studies) and studies with less than 100 subjects (17 studies). After the exclusion, we found that pooled prevalence was 8.3% (95%CI, 6.6-10.4%) with significantly high heterogeneity among studies (I^2=94%, p<0.01) (Supplementary figure 3).

Prevalence of malnutrition by MNA

The pooled prevalence of malnutrition based on MNA was 6.1% (95% CI 3.8-9.4) (Figure 2). The prevalence was highest in the inpatient setting (21.1%; 95% CI 16.1-27.1) and lowest in the community setting (5.6%; 95% CI 3-10.3). None of the studies had a high risk of bias. The highest prevalence was among studies in the Northern region (11.2%; 95% CI 3.6-29.9). There was significantly high heterogeneity among studies (I^2=96%, p<0.01). After we performed sensitivity analysis by excluding subject numbers below 100, the pooled prevalence was 5.3% (95% CI 3.4-8.4) (Supplementary figure 4).

Prevalence of malnutrition by MNA-SF

The pooled prevalence of malnutrition based on MNA-SF was 5.7% (95% CI 4-8.1) (Supplementary figure 5). Three studies were too small to test for heterogeneity.

Prevalence of malnutrition by other assessment tools

Because there were insufficient studies using other tools, we were unable to perform a meta-analysis of the prevalence of malnutrition by those methods. The prevalence of malnutrition assessed by skinfold thickness was 32.9% in community settings and 57.6% in outpatient settings, by mid-arm circumference was 3.1% in community settings, and 61.0% in outpatient settings, and by calf circumference was 30.2% in community settings. By nutrition assessment tool, the prevalence of malnutrition by SGA was 39.1% in the hospitalized setting.

In studies that used biochemical data to assess malnutrition, the prevalence of low albumin was 0% in a community setting, 19.8% in an outpatient setting, and 23.6-85.9% in the hospitalized setting. When assessed by a total lymphocyte count below 1500, the prevalence was 56.4% in the hospitalized setting. By definition of low hemoglobin, there were 33.6%-80.9% in hospitalized setting and 74.2% (from one study) in an outpatient setting, similar to low hematocrit [33.9-81.8% and 73.6% (from one study) in hospitalized and outpatient, respectively]. One study defined cholesterol below 160 mg/dL as malnutrition and reported the prevalence to be 39.8% in hospitalized patients.

Prevalence of at-risk of malnutrition by MNA

The pooled prevalence of at-risk of malnutrition based on MNA is 42.6% (95% CI 36-49.4). (Supplementary figure

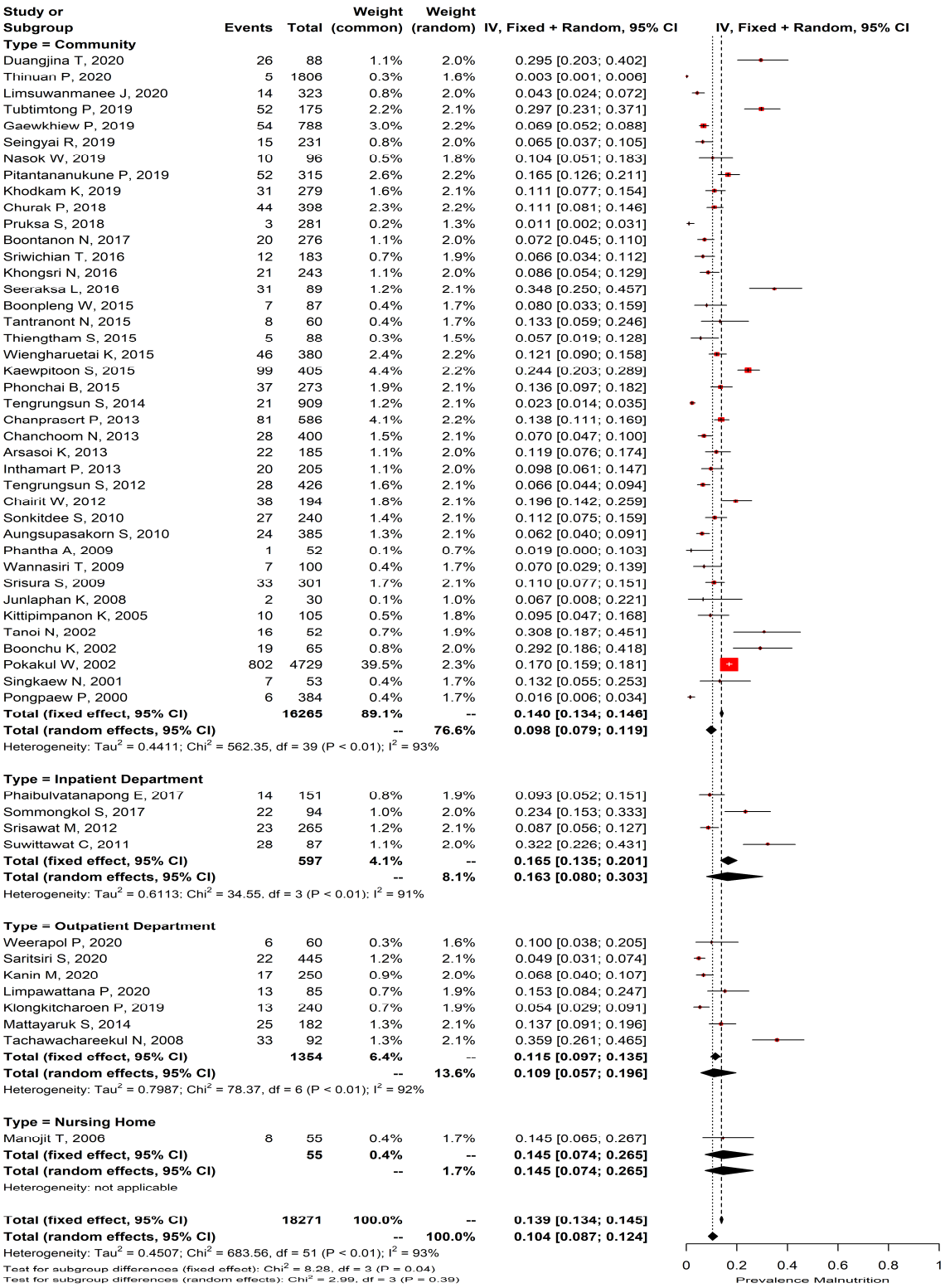

Figure 1. Forest plot of studies of malnutrition in Thai older people by BMI.

6) There was significantly high heterogeneity among studies (I^2=97%, p<0.01). After excluding studies with fewer than 100 subjects, the prevalence was 41.0% (95% CI 34-48.4) (Supplementary figure 7).

Prevalence of at-risk of malnutrition by MNA-SF

The pooled prevalence of at-risk of malnutrition based on MNA-SF was 37.8% (95% CI 32.1-44.0) with significantly high heterogeneity among studies (I^2=92%, p<0.01) (Supplementary figure 8). After excluding studies with fewer than 100 subjects, the prevalence was 35.5% (95% CI 30.3-41.1) (Supplementary figure 9).

Prevalence of at-risk of malnutrition by other assessment tools

Because there were insufficient studies, we were unable to perform a meta-analysis of the prevalence of at-risk of malnutrition. The prevalence was 32.6%-54.3% by MST, 46.3-76.2% by NRC, and 53.1%-92.5% by NRS. All of these studies were done in the hospitalized setting.

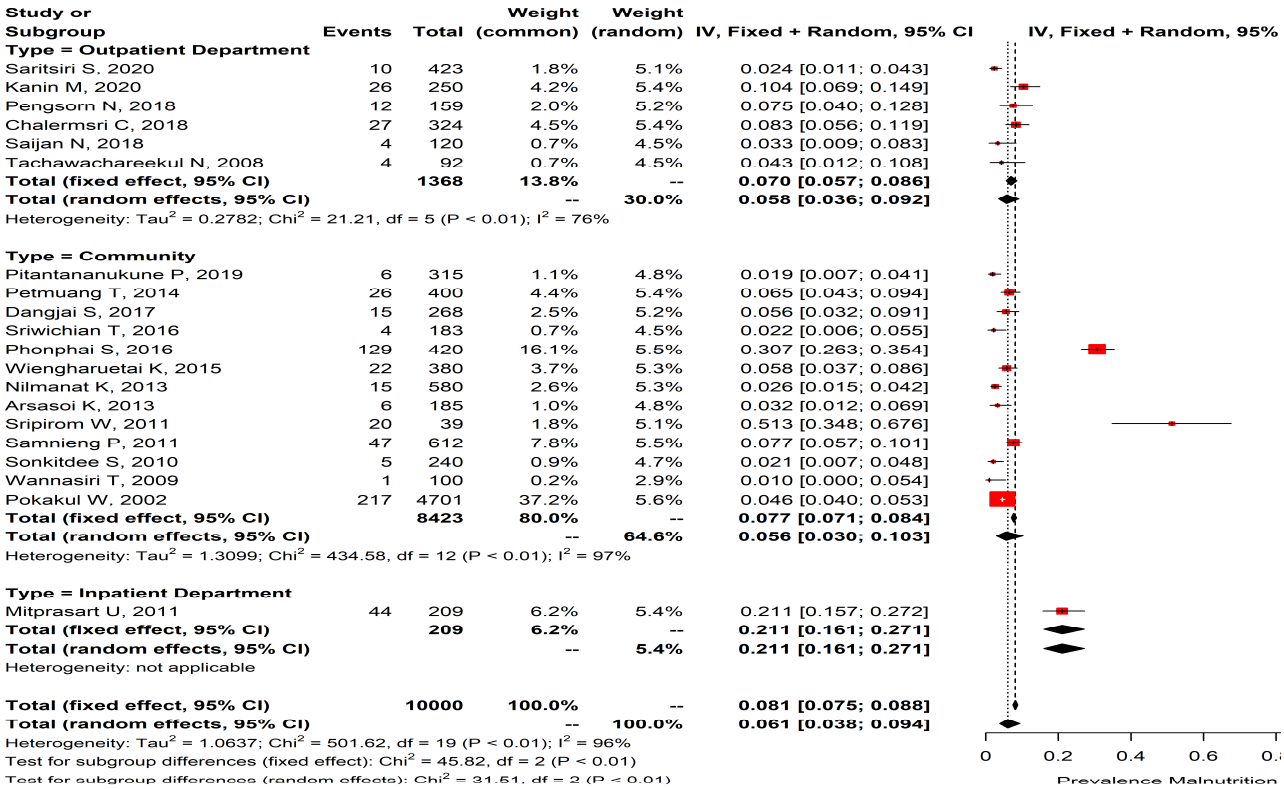

Figure 2. Forest plot of studies of malnutrition in older Thai people by MNA.

Associated factors of malnutrition

Malnutrition is more prevalent in rural[27] than urban communities. Malnutrition is associated with female gender,[23,29,88] advanced age,[23,27,29,33,40,41] low education,[29,88] single marital status,33 and living alone.[40] Comorbidities[40, 88] such as dementia,[29] depression,[29,41,46,78] cerebrovascular disease,[29] and more severe comorbidities (defined by a higher score of Charlson Comorbidities Index).[29] Eating-related problems related to malnutrition include appetite disorder,[33] number of teeth,[23,67,76] functional dentition,[36] mouth dryness,[88] and avoidance of eating vegetables and fruits.[88] In addition, geriatric conditions including functional decline,[29,40,78] frailty,[89] and polypharmacy[29] are associated with malnutrition.

Weight loss, poor appetite, swallowing and chewing difficulties

Though weight loss and poor appetite were included in the MNA, few studies reported each item separately. Most of the studies using MNA reported the total score of MNA and MNA-SF. Report of weight loss item was presented in only nine studies in which some studies defined weight loss as kilogram with different cutpoints and some defined as the percent of weight loss.[23,34,56,73-75,87,89,90] Using the loss of at least 3 kilograms as a cutpoint, the proportion of weight loss was 5-7.1% in the community-dwelling older people.[34,73,74,90] Using at least a 1-kilogram cutpoint, the proportion of weight loss ranged from 10.0-62.2% in the community-dwelling older people.[34,73,74,90] Using at least 5% of weight loss in the previous year, the reported prevalence of weight loss was 5.8-18.3% in the community-dwelling older people.[87,89] The prevalence of weight loss was reported in 83.4-85.2% in those with cancer and current hospitalization.[56,75]

Poor appetite and poor oral intake were reported in 7 studies as isolated items ranging from 1.92-36.2% in community-dwelling older people.[23,25,33,38,48,57,90] The swallowing difficulty was reported in 3.8-10% in the community, 15.7-43.1% in an in-patient, and 23.6% in a nursing home.[38,41,48,50,57] Difficulty in chewing was reported in 17.3-56.7% in a community setting, 39% in an OPD setting, 57.4-84.3% in an IPD setting, and 67.3% in a nursing home setting.[38,41,48-50,57,60,80,88]

Food choices of older people in Thailand

Several factors in older people's lives can affect food preferences and choices. The individual factors, nutritional knowledge, ethnicity, beliefs, and ecosystems factors; economic system, society, food safety, food sources can all contribute to individual food choice and habits in the older person.[96]

In this study, we found that both individual and ecosystem aspects are associated with under-nutrition in older people. Nutrition knowledge is one of the important factors that have been found associated with nutrition status in older people in Thailand.[25,47,66] Avoidance of certain food such as chicken, bamboo shoots, ferment foods, green hatching during sickness, and meat, catfish, or eels is due to personal belief.[48,71] Culture and ethnic background also influence the food choices; for example, Mon people prefer to eat a high-fiber diet and some tropical vegetables like Okra.[73] Even though older people in Thailand have relatively low incomes, most of them can access enough food.[25,28,37,38,48,60,71,82] Most older people in Thailand live and share meals with family, so the problem

of skipping meals that are related to social isolation is less common.[96] In terms of food sources, older people and families usually buy raw material from a local market or their farm,[37,51] and prepare foods at home rather than buying ready-to-eat, canned, or frozen food.[51,73] Furthermore, the cleanliness of food is a big concern of older people[28] and possibly correlates with the BMI of older people.[85]

The component of each meal (Table 2) and the quantity of food intake are also important factors that contribute to the under-nutrition problem.

Publication bias

Publication bias can affect the results of the meta-analysis and so a funnel plot was applied. There was publication bias among studies in the BMI tool (p=0.001) (Supplementary figure 10). However, studies using the MNA tool showed no publication bias (p=0.52) (Supplementary figure 11).

DISCUSSION

This meta-analysis included 71 studies and the pooled prevalence of malnutrition among older people in Thailand was 10.4% by BMI, 6.1% by MNA, and 5.7% by MNA-SF. Prevalence of at-risk of malnutrition was 42.6% by MNA and 37.8% by MNA-SF. The mean age of participants in each study ranged from 65.5 to 78.3 years. Poor appetite and chewing difficulty were common. Physiological, psychological, and socio-economic factors were associated with malnutrition.

BMI is the most commonly used tool in all settings, probably due to its simplicity and convenience. Other anthropometric measurements such as skinfold thickness, which reflects body fat, and muscle circumference of the mid-arm or calf, which reflects muscle mass, is not always reliable in Asian populations where reference values have not been established. Therefore, these methods are not suitable for assessing malnutrition in Thai people. In addition to BMI, MNA-SF and MNA are also commonly used for screening and assessment for malnutrition in older Thai people. Other validated tools include MUST, MST, NRS-2002, and SGA, none/few of which were used in studies in older Thai people and preventing meta-analysis. Some studies used biochemical markers to indicate malnutrition, of which serum albumin was the most commonly used. However, serum albumin is not appropriate in acute illness as it may be falsely elevated due to dehydration. Moreover, serum albumin may decrease in other causes such as inflammation, infections, trauma, heart failure, edema, liver dysfunction, and nephrotic syndrome. Lymphocyte, hemoglobin, or hematocrit are not specific to malnutrition and are also affected by other conditions.

The pooled prevalence of malnutrition assessed by MNA may be lower than with other tools as it contains the group of at-risk for malnutrition separately from malnutrition. Moreover, the MNA tool evaluates not only anthropometric measurements including BMI, but also overall physical health, neuropsychological problems, and self-evaluation of nutrition and health status. It can be difficult to get information from older people, especially those with cognitive impairment. In our study, the pooled prevalence of malnutrition by BMI was 10.4%, higher than the prevalence by the MNA tool (6.1%) and MNA-SF (5.7%). However, the prevalence of being at-risk of malnutrition by MNA and MNA-SF were 42.6% and 37.8%, respectively. The MNA was developed in western countries and the cut-off levels of some items, such as BMI below 19 kg/m^2, mid-arm circumference below 21 cm, and calf circumference below 31 cm, might be inappropriate in Asian populations. Our results suggest that the MNA may require further study to identify a more appropriate cut-off value to evaluate older Thai people for malnutrition.

The lowest prevalence of malnutrition was among community-dwelling older people which was 9.8% using the BMI and 5.6% using the MNA. The highest prevalence was among hospitalized older people, 16.3% and 21.1% by BMI and MNA, respectively. Older people in hospitals are more vulnerable to nutritional disorders, and acute illness leads to anorexia in older people. On the other hand, nutritional disorders might increase the risk of hospitalization due to infection, frequent exacerbation of chronic lung disease, or poor muscle mass leading to falls and fracture. A nutritional assessment should be performed during hospital admission, at least once weekly during a short hospital stay, every 15 days for rehabilitation care, and once monthly during long-term care.[97] Nutritional intervention as part of a multidisciplinary care team should be focused on hospitalized older people.

The higher malnutrition prevalence in rural[28] than urban communities aligns with previous research.[98] This may be because rural areas may have poorer dietary quality and micronutrient deficiency, leading to poor nutritional status. The current guidance by the Integrated care for older people (ICOPE) guidance is launched for primary care health workers to provide initial screening and care pathways in community-dwelling older people.[99] The first step is the screening by 2 questions regarding weight loss of 3 kilograms over the last 3 months and loss of appetite. The second step is the assessment of nutritional status by more comprehensive tools such as MNA, DETERMINE, or SNAQ. This recommendation is similar to the current Thai guidance for a community screening in the initial step by the older people themselves, family caregivers, or village health volunteers and followed by the MNA-SF/MNA in the second step by the healthcare professional.[100] This study may provide an information about malnutrition in older Thai people, which can be a reference data for the future study in aging population.

The varied reported prevalence among studies is likely from 3 reasons. First, the population characteristics in the studies are varied such as mean age (young old vs very old), community/ outpatient/ in-patient setting, and comorbid illness. Even in the same patient setting such as hospitalized patients, there were still differences in population studied in that patients were recruited from medical ward, new cancer patients scheduled for new chemotherapy session, and patients receiving urgent abdominal surgery. Second, the subjects included in the research usually had no communication or cognitive problems, did not require mechanical respiratory support or were not critically ill, and were able to be weighed. This inclusion and exclusion criteria could result in selection bias of relatively better health of subjects compared to those excluded.

Table 2. Food consumption behavior in older people in Thailand

Study	Population	Number of subjects	The most frequent consumption food products reported						Family and social
			Carbohydrate	Protein	Fat	Vegetable	Fruit	Frequency (meal/day)	
Boonchu K, 2002[25]	Community dwelling elders	65	Sticky rice	Fish	Fat from animal products	Cabbage, green onion, ivy gourd	Mango	3 times	*NA*
Inthamart P, 2013[37]	Community dwelling elders	205	Sticky rice	Milk and fish	Fat from animal products and vegetable oil	Local vegetable	General fruits	*NA*	Eat with family
Manojit T, 2006[48]	Elder nursing homes	55	Plain rice	Nonspecific meat and soy milk	Fat from animal products	Bog choy, water spinach, cabbage	Banana, orange papaya	3 times	Eat with company
Nasok W, 2019[51]	Community dwelling elders	96	Sticky rice	Nonspecific meat	Fat from animal and plant products	Green vegetable	*NA*	*NA*	*NA*
Pruksa S, 2018[64]	Community dwelling elders	281	Sticky rice	Fish	Vegetable oil	General vegetables	Orange, papaya, watermelon, rose apple, banana, pineapple	3 times	Eat with family
Suwittawat C, 2011[79]	Hospital	87	Sticky rice and plain rice	Fish	*NA*	General vegetables	General fruits	3 times	*NA*

NA: not available.

Therefore, there might be the underestimation of the true prevalence. Third, the criteria and screening tools used were different. The two most commonly used tools were BMI and MNA. This, in fact, should lead to a comparable prevalence. However, as older people tend to have decreased height either from osteoporosis or malalignment of spinal curvature resulting in falsely high BMI. Many older adults cannot stand or be weighed which limited the assessment of BMI in some places. Moreover, body weight contains both body fat and fat free body mass. People with normal or high BMI might have associated muscle loss and decline in muscle function called "sarcopenic obesity" which also have clinically important negative health outcomes. The MNA-SF also contains the BMI item. Though there is the option of assessing calf circumference (CC) in case of the difficulty to measure BMI, there was a report that BMI-incorporated MNA-SF showed higher accuracy as compared to CC-incorporated MNA-SF.[101] Moreover, in older people where there are problems with communication, consciousness or cognitive function, some of the items might not be able to score as they need recollection of information.

Older people in community-dwelling and OPD settings who had malnutrition had a higher proportion of sarcopenia as demonstrated by the Bioelectrical Analysis of body composition[29,43,86,102] and a higher proportion of pre-frailty, frailty, and cognitive frailty.[87,89,103] Both sarcopenia and frailty could lead to further negative health outcomes. Several factors associated with food choices and dietary practice include physical health, mental health, society, environment, and economic systems. Other issues leading to varying food choices are food preference (such as ethnic food preference, ready-to-eat foods), trust and concerns in the food market, and food safety.[96]

In other studies, especially in western countries, undernutrition is associated with decreased food intake from social isolation or psychological status. In contrast, Southeast Asian older people live with their families, providing food and taking care of them. In addition, the components of each meal and the number of daily meals seem to be expected (Table 2). Collectively, it seems that availability and quality of food may not be the main factors contributing to malnutrition status in these populations. Instead, the quantity of food intake itself could play a key factor in malnutrition. Even though the Ministry of Public Health provides information about nutrition and how to eat properly, the instruction may not be practical in real life. Moreover, older people usually have underlying diseases that limit specific food intake. It makes sense that loss of appetite is associated with malnutrition status and possibly be a warning sign of under-nutrition; however, the lack of patient's appetite data in many studies and standard measurements to evaluate appetite makes this relationship unclear.

In terms of availability, access, utilization, and stability, food security was surveyed in older people and found to be fair to good.[24,28,85] However, almost half of older people still had a poor to a fair level of food literacy, attitude toward Thai food-based dietary guidelines, and dietary habits following the guideline.[39,45,47,66,83] In addition, the primary resource the older people reported to receive the knowledge about food were from healthcare professionals, whereas public media was a less common resource.[25] This highlights the universal approach to public education. Several malnutrition-related factors could be modified. Poor appetite was common in older people, and some causes of poor appetite such as medication, depression, oral health, medical illness could be improved. The primary cause of the chewing difficulty is poor oral health, and dentation should be routinely screened and corrected. Food literacy, attitude toward national food guidelines, and dietary habits should be focused. Action toward other resources of appropriate dietary practice on top of healthcare professionals should be expanded. We summarize the conceptual framework of determinants and consequences of malnutrition in Figure 3.

Strengths

This is the first national estimate of the pooled prevalence of malnutrition and at-risk of malnutrition among older people in Thailand. We included all studies that used validated tools for screening and assessment of malnutrition with both published and unpublished data, and without language restriction. The subgroup analysis revealed different prevalences based on population characteristics, study setting and region. We also performed a systematic review of associated factors of malnutrition in older Thai people.

Limitations

Due to high heterogeneity, the random effect model prevalence should be used with caution. Interpreting the pooled prevalence should take this heterogeneity into account. Publication bias was detected in the studies using BMI, but not with the MNA tool. However, because data from multiple settings were included, the findings of this study may be generalized to the older people in both community and hospitalized settings.

Conclusions and implications

The pooled prevalence of malnutrition among older people was 6-10%, depending on assessment methods and study settings. Healthcare professionals should use appropriate malnutrition screening and assessment tools in the appropriate context. Older people should be weighed and BMI should be checked and monitored during every visit in general practice. These estimates can be used by policymakers and public health workers for controlling malnutrition and intervention strategies. Nutritional screening should focus on people with associated factors of malnutrition. Hospitalized older people are at greater risk of malnutrition. Several risk factors for malnutrition are modifiable. Developing a standard score for evaluating appetite in older people should become another interesting topic to better evaluate and manage older people with malnutrition problems. A routine check of oral health, chewing, and swallowing problems would lead to early corrective intervention for malnutrition. If malnutrition is detected early and treated promptly, its effects can be arrested and reversed, reducing illness and mortality.[104] We propose that the policy focus on giving a practical knowledge of nutrition that relates to the context of local culture. Multiple formats of delivering knowledge and appropriate dietary habits should be targeted.

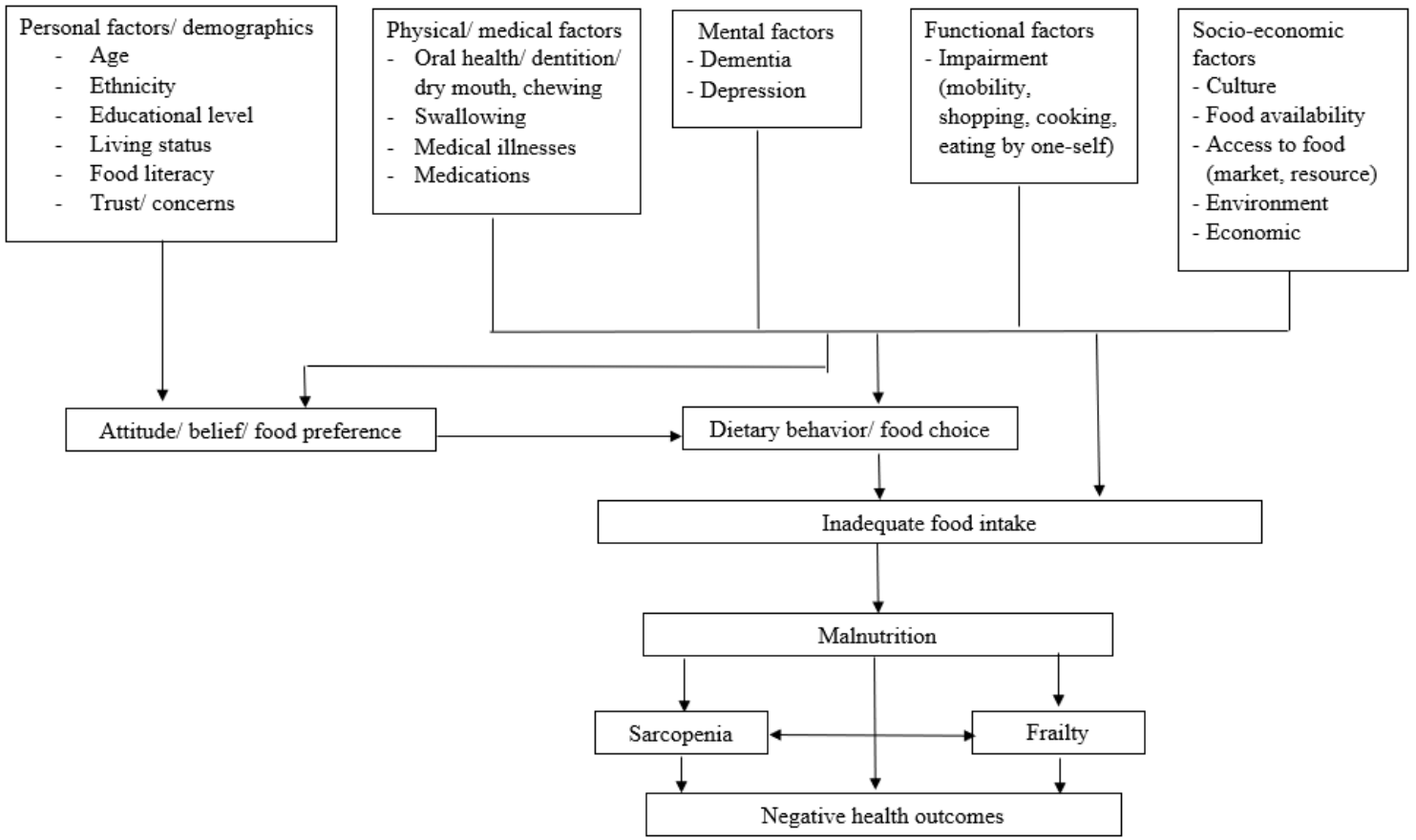

Figure 3. Conceptual framework for determinants and consequences of malnutrition in older people.

AUTHOR DISCLOSURES

The authors declare no conflict of interest.

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Original Article

Central obesity in low BMI as a risk factor for COVID-19 severity in South Indians

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Background and Objectives: South Asians are known to have excess adiposity at a lower body mass index, with truncal fat accumulation. Whether this confers higher risk to develop severe COVID-19 is not known. This study evaluated body mass index, body fat mass and waist circumference as risk factors for COVID-19 severity and its progression, in South Asian adults. **Methods and Study Design:** Details of COVID-19 patients (19-90 years) were obtained prospectively, along with weight, height, waist circumference and body fat mass assessed by bioelectrical impedance analysis. Binomial logistic and Poisson regression were performed to test associations between waist circumference, body fat mass and body mass index to evaluate the adjusted OR or relative risk for disease severity at admission and length of stay. **Results:** After adjusting for age, sex, height and co-morbidities, body mass index >23 kg/m^2 (adjusted OR 2.758, 95% CI 1.025, 7.427), waist circumference (adjusted OR 1.047, 95% CI 1.002, 1.093) and body fat mass (adjusted OR 1.111, 95% CI 1.013, 1.219) were associated with a significant risk for disease severity at admission, while only waist circumference (adjusted relative risk 1.004, 95% CI 1.001, 1.008), and body fat mass (adjusted relative risk 1.011, 95% CI 1.003, 1.018), were associated with a significantly longer length of stay. **Conclusions:** Body mass index, at a lower cut-off of >23 kg/m^2, is a significant risk factor for COVID-19 disease severity in the group of patients studied. The waist circumference and body fat mass are also good indicators for both severity at admission and length of stay.

Key Words: bioelectric impedance analysis, body mass index, body fat, central obesity, COVID-19, SARS-CoV-2, visceral adiposity, waist circumference

INTRODUCTION

Obesity and particularly central obesity as defined by body mass index (BMI) and waist circumference (WC), respectively, have consistently emerged as robust risk factors for COVID-19 severity at admission and outcome. For example, a recent UK study reported a dose-response increase in risk for COVID-19 severity at BMI >25 kg/m^2, as well as a higher risk associated with central obesity.[1] However, it is likely that the BMI-associated risk for severity may appear much earlier in South Asians, who are thought to have higher adiposity at lower BMI with greater truncal fat accumulation.[2] It is then useful to additionally test the body fat mass (FM%) and WC (as a proxy for central or visceral adiposity) as direct risk factors. The present study evaluated the association between BMI, FM% and WC with COVID-19 severity at admission, and its progression as length of stay (LOS), in adult patients from a tertiary care hospital, with a BMI range that was similar to earlier reports.[1,3]

METHODS

Admitted patients with COVID-19, aged between 19-90 years, were recruited into the study from a tertiary care hospital, after institutional ethics committee approval (IEC reference number 175/2021) and their written in-

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formed consent. Pregnant women, patients admitted in intensive care (ICU), on inotropic support, dialysis, or who were unable to maintain posture during the height measurement, were excluded. Demographic details, clinical parameters (e.g., oxygen saturation, respiratory rate), inflammatory markers like D-dimer and C-reactive protein (CRP) and disease severity score at admission were obtained from patient records. Disease severity at admission was classified as mild, moderate and severe, based on published national guidelines.[4] The hospital course of recruited participants included details on LOS, oxygen requirement, days on oxygen, ventilatory support, need for ICU support and mortality.

Body weight and height were recorded to the nearest 0.01 kg and the nearest 0.1 cm respectively. WC was measured at the mid-point between the lower edge of the rib cage and the iliac crest using a non-metal, non-stretchable measuring tape to the nearest 0.1 cm. Triplicate measurements by two investigators were averaged, with a CV <2%. Bioelectrical impedance was measured at 4 frequencies (BIA, Quadscan 4000, Bodystat Ltd, British Isles) with a standard quality protocol,[5] to calculate FM% by equations provided by the manufacturer. Since regular electrode supply was disrupted due to the pandemic, preliminary measurements were made to evaluate whether electrocardiogram (ECG) electrodes could be used for this purpose. Simultaneous measurements by ECG and the Bodystat electrodes showed that the average difference between the measurements was 0.2 (95% CI, -0.1, 0.4) for FM%, with a good correlation (R^2=0.98) between electrodes, and therefore, both types of electrodes were used.

Multivariate associations of severity at admission, hospital course parameters, and inflammatory markers were explored with WC, BMI and FM%, adjusting for age (>45y), sex, height (except in the BMI association), diabetes (DM) and hypertension (HTN). BMI was used as both, a continuous and categorical variable (with a cut-off of ≤23 and >23 kg/m^2), based on the action point suggested for South Asians.[6] However, WC and FM% were only analysed as continuous variables, as there were not adequate numbers available for applying separate sex-based cut-offs for either. For severity at admission, moderate and severe degree of disease were combined into a single category of 'severe'. Binomial logistic regression was performed for severity at admission, considering 'mild' disease as reference. For the skewed distribution of serum CRP at admission, a log linear regression was used, and its slope reported. A Poisson regression model was used for LOS, because of a count response, with effect size in terms of relative risk (RR). An observed false positive rate of <5% was considered statistically significant. R statistical software, version 4.1.0 (R Core Team, 2021, Vienna, Austria), was used.

RESULTS

COVID-19 patients (n=172) admitted in a tertiary care hospital between 1st June 2021 to 15th June 2021, were studied. Their range of BMI was 16.8 to 45.3 kg/m^2, and their demographic characteristics, anthropometry, BIA, oxygen saturation, inflammatory markers at admission and hospital course of the patients are summarised in Table 1. The sex ratio was skewed toward males (65%). The most common associated co-morbidities were DM (57%) and HTN (27%). About a third of the patients required either ICU care or step-down intensive therapy support during their hospital stay, while one fourth were discharged on home oxygen; 3 deaths occurred. WC was significantly and positively associated with BMI and was stronger (r=0.68, 95% CI 0.58-0.76) when BMI was >23 kg/m^2, while FM% showed a significant positive correlation only with BMI >23 kg/m^2 (Figure 1a and 1b).

In multivariate linear regression, FM%, WC and categorical BMI >23 kg/m^2 emerged as significant risk factors for disease severity at admission after adjusting for age, sex, height, DM and HTN (Table 2). BMI as a continuous variable was not associated with severity. For every unit increase in WC or FM%, the AOR of having 'severe' COVID-19 at admission was 5% or 11% higher, respectively. The AOR was nearly 3 times higher for severe COVID-19 at admission with BMI >23 kg/m^2.

Every 10 cm increase in WC or 10 unit change in FM% had a risk of an extra 9-hour or ~1 day in LOS respectively (Figure 2a and 2b). However, BMI as either a continuous or categorical variable was not associated with LOS. Serum CRP was significantly and positively associated

Table 1. Demographics, anthropometry, and clinical parameters of patients

Parameter	Mean (SD)	Median (Q1, Q3)
At recruitment		
Age, y	51 (13)	51(41, 60)
Anthropometry		
Weight, kg	70.3 (14.7)	68.8 (60.6, 78.7)
Height, cm	163 (8.7)	164 (156, 169)
WC, cm	93.1 (11.4)	93.0 (86.1, 100)
BMI, kg/m^2	26.4 (5.1)	25.8 (22.9, 28.8)
FM, %	33.4 (9.4)	30.5 (25.9, 40.3)
At admission		
SpO_2, %	87 (10)	89 (82, 94)
CRP, mg/mL	8.5 (7.9)	6.2 (2.3, 11.9)
D-dimer, ng/mL	725 (1238)	384 (229, 626)
Hospital course		
Length of hospital stay, days	14.6 (9.0)	12 (8, 20)
Days on oxygen	12.8 (10.4)	11 (5, 19)

BMI: body mass index; CRP: C-reactive protein; FM: body fat mass; WC: waist circumference; SpO_2: oxygen saturation.

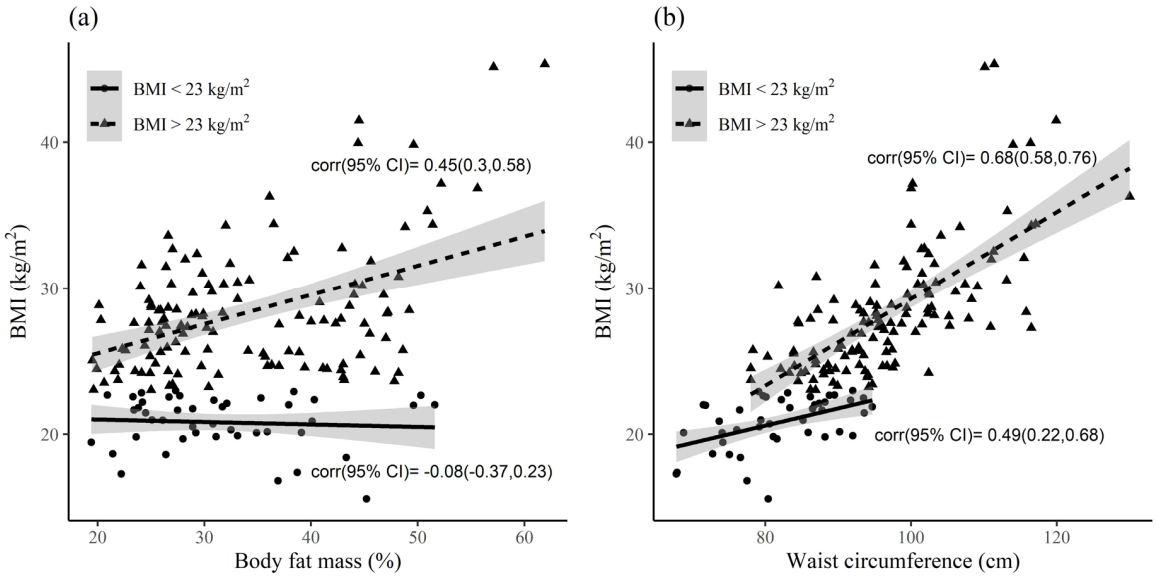

Figure 1. Association of FM% and WC with BMI below and above 23 kg/m^2. Regression lines were fitted for BMI ≤23 (solid line) and >23 kg/m^2 (dashed line) in relation to FM% and WC. Shaded portion is the 95% confidence band. (a) FM% in relation to BMI (b) WC in relation to BMI. BMI: body Mass Index; FM%: body fat mass; WC: waist circumference.

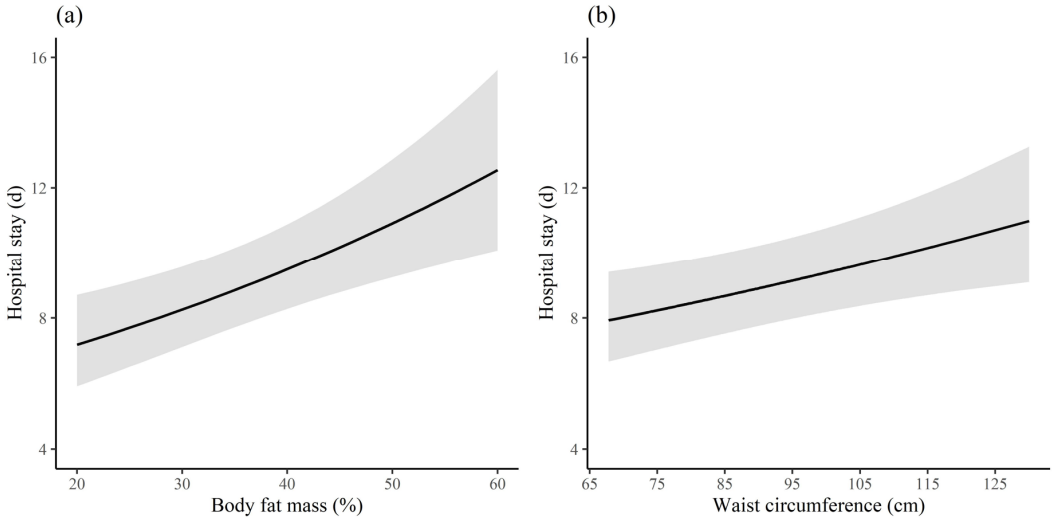

Figure 2. Adjusted Poisson regression predicting LOS by FM% and WC. The Poisson regression model was adjusted for sex, age (>45 y), height, diabetes and hypertension. The solid line signifies the mean LOS in response to increasing FM% and WC. Shaded portion is the 95% confidence band. (a) LOS in relation to FM% (b) LOS in relation to WC. LOS: Length of hospital stay (days); FM%: body fat mass; WC: waist circumference.

Table 2. Risk factors for 'severe' COVID-19 at admission and length of hospital stay†

	'Severe' versus mild	LOS	CRP, mg/mL
	AOR (95% CI)	ARR (95% CI)	Slope (95% CI)
WC, cm	1.047 (1.002, 1.093)*	1.004 (1.001, 1.008)*	0.019 (0.000, 0.037)*
BMI, kg/m^2	1.089 (0.982, 1.207)	1.002 (0.994, 1.011)	0.015 (-0.030, 0.059)
BMI > 23 kg/m^2	2.758 (1.025, 7.427)*	1.050 (0.960, 1.150)	0.146 (-0.326, 0.618)
Body Fat Mass, %	1.111 (1.013, 1.219)*	1.011 (1.003, 1.018)**	0.045 (0.006, 0.084)*

AOR: adjusted odds ratio; ARR: adjusted relative risk; LOS: length of hospital stay; CRP: C-reactive protein; WC: waist circumference.
†Binomial logistic regression was performed for COVID-19 severity at admission ('severe' versus mild); Log linear regression was used for CRP; Poisson regression model was used for LOS; adjusted by stepwise regression for age (>45 y), sex, height, diabetes (DM) and hypertension (HTN).
*p<0.05, **p<0.01.

with WC and FM% but not with BMI (Table 2). There was no association of any other anthropometric measure with other characteristics such as days on oxygen, medications used, need for intensive care, other inflammatory markers or discharge outcome.

DISCUSSION

In the present study, the BMI, even at a relatively lower cut off of 23 kg/m^2 showed a ~3 times higher odds of COVID-19 severity at admission, in adult patients from a tertiary care hospital. While a similar risk has been noted

earlier in a UK group of patients, this magnitude of risk was noted at a much higher BMI range of 35-39.9 kg/m^2.[1] The finding points towards a higher risk for COVID-19 severity at a lower BMI but with a relatively higher fat accumulation, especially in the truncal region.

In keeping with the framework of excessive fat accumulation, particularly truncal fat, FM% and WC were also significant risk factors for COVID-19 severity at admission and LOS. The effect on LOS has implications for healthcare capacity, personnel utilization and costs, particularly in resource-poor settings. However, BMI did not emerge as a significant risk factor for disease severity at admission or LOS in continuous analyses probably because of the non-linear relation that existed between FM% and BMI, where the FM% variability was high in the BMI ≤23 kg/m^2 category, with no correlation. However, there was a significant correlation between FM% and BMI in the >23 kg/m^2 category (Figure 2a), with the attendant higher AOR for severity. In addition to this, WC significantly correlated across the BMI range, particularly for BMI >23 kg/m^2 (Figure 2b), suggesting that fat mass accretion was probably centrally located.

The WC is a simple method of measuring visceral adiposity and has been shown to be associated with COVID severity and death.[1,7] A retrospective analysis of a UK cohort (n=489,769 adults) showed that with every 10 cm increase in WC (measured in 2006-2010) the unadjusted odds of COVID severity significantly increased by 35% (p<0.001).[1] In comparison, the AOR was nearly two-fold higher in the present study, which emphasizes the risk associated with central fat accumulation in relatively thin people. In a retrospective analysis on 215 hospitalized patients with COVID-19, WC (≥102 cm for men and ≥82 cm for women) showed a significant association with chest X-ray derived severity scores, rather than the BMI, which is similar to the findings of the present study.[8] Human visceral adipose tissue is implicated in severe COVID-19 pathogenesis because it is proinflammatory[9] and has a higher gene expression for Angiotensin Converting Enzyme 2 (ACE2) receptor.[10] Recent studies have demonstrated how SARS-CoV-2 can infect adipocytes in *in vitro*[11] as well as *in vivo* settings and that macrophages and preadipocytes within the adipose tissue participate in replication of the virus and in inflammation.[12] This is corroborated here with the significant association between FM% or WC with the inflammatory marker, serum CRP, at admission.

The strength of this study is the additional measurement of body composition and WC with BMI in hospitalised COVID patients. Drawbacks include study in a single hospital that is not generalizable, cross-sectional nature, with anthropometry and FM% measurements at different points of hospital stay. The confounding effect of different treatment protocols and premature discharge due to financial or social constraints are other limitations to data interpretation.

In conclusion, the risk of COVID-19 disease severity occurs at a much lower BMI cut-off in South Indians and could be considered for triaging at admission in this group of individuals. WC and FM% also show potential as good risk indicators that can be used for both severity at admission and LOS, and further studies on these lines are required to support the suggestion. The findings also highlight the need for public health interventions that focus on a better body composition and waist size reduction to buffer the impact of the pandemic.

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AUTHOR DISCLOSURES

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Original Article

Genetic susceptibility to cow's milk allergy in Chinese children

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Background and Objectives: Cow's milk allergy (CMA) is the most common food allergy in young children. Previous studies have reported that single-nucleotide polymorphisms (SNPs) are associated with CMA. The extent to which SNPs contribute to the occurrence of CMA is unknown. The purpose of this study was to investigate the independent relevance of genetic predisposition to CMA in Chinese children. **Methods and Study Design:** 200 infants with CMA and 799 healthy controls aged 0–12 months were included. Five previously identified genetic variants (rs17616434, rs2069772, rs1800896, rs855791 and rs20541) were genotyped. Logistic regression was used to analyze the genetic associations or their interactions with a family history of allergy on CMA. **Results:** Among the five SNPs, only IL10 rs1800896 was significantly associated with CMA (odds ratio (OR) 1.60, p=0.042). Each 1-risk allele increase in the genetic risk score (GRS) was suggestively associated with an 11% higher risk of CMA (1.11: 0.99–1.27, p=0.069) and a 45% increased risk of CMA in the GRS high-risk group compared to the GRS low-risk group (1.45: 1.02–2.06, p=0.037). Furthermore, parental allergy also increased the risk of CMA among children (1.87: 1.46–2.39, p<0.001). Importantly, parental allergy exacerbated the genetic effect on the risk of CMA. **Conclusions:** The rs1800896 variant in the IL-10 gene is associated with CMA in Chinese children. In addition, the GRS had an interaction with parental history of allergy, implying that genetic risk for CMA was exacerbated among those with parental history of allergy.

Key Words: cow's milk allergy, single- nucleotide polymorphism, genetic susceptibility

INTRODUCTION

Cow's milk allergy (CMA) is the most common food allergy in infants and young children, with an estimated incidence ranging between 2% and 3.5%.[1-3] CMA can occur in infants who were exclusively breastfed or those who received mixed feeding (with introduction of milk protein).[3] There are 3 types of inflammatory mechanisms that can mediate CMA: "acute-onset" immunoglobulin E (IgE)-mediated allergies, "delayed-onset" non-IgE cell-mediated allergies, and mixed-type-mediated allergies.[4] A total of 82.5% of CMA babies develop allergic reactions in the first 3 months of life. The etiology of CMA is complex, and genetic risk factors have a strong influence. If one parent is allergic, the risk of allergic reactions rises to approximately 20% to 30%.[5] If both parents are allergic, their children have an approximately 40% to 70% chance of also developing it.[6] Patients with CMA and other food allergies have a heterogeneous clinical presentation, and the estimated heritability of food-specific IgE ranges from 0.15 (cow's milk) to 0.35 (wheat).[7,8] Although up to 90% of affected infants naturally develop tolerance to cow's milk proteins by 5 years of age,[9] children with CMA early in childhood seem to have a higher risk of developing asthma and other allergic diseases later in life. CMA is caused by the combined effect of genetic susceptibility and environmental influences, and exploring the genetic characteristics and related genes of CMA may provide valuable information for intervention and treatment.

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Several studies have addressed the association of gene polymorphisms with CMA and have identified candidate genes, some of which are also associated with asthma. Peter et al analyzed six SNPs that have been described previously in relation to allergic diseases and found two SNPs, rs17616434 and rs2069772, to be significantly associated with CMA.[10] Rs17616434 is located near a cluster of Toll-like receptor (TLR1, 6, 10) genes. TLR is a member of the interleukin-1 receptor (IL-1R) superfamily and is a highly conserved molecular family in human evolution. It plays a key role in the innate immune response by identifying pathogen-associated molecular patterns (PAMPs).[11] Rs2069772 was previously reported to be associated with allergic rhinitis and is located near the IL2 and KIAA1109 genes.[12] In 2006, Ulla Christensen et al. reported for the first time that in the Danish population, IL-2 can be used as a candidate gene for asthma and found that two polymorphic sites in the IL-2 gene are related to mediating allergic diseases.[13] The other gene, KIAA1109, is known to be involved in celiac disease, a disease characterized by a strong immunological response to food proteins (gluten), found in wheat, rye and barley.[14] As an immunomodulatory factor, IL-10 plays an important role in the production of Th1 and Th2 cells and the secretion of cytokines. Studies have shown that the secretion of IL-10 is affected by genetic factors, and the level of IL-10 in the human body will directly affect the susceptibility and severity of diseases such as food allergies. Therefore, research on IL-10 gene polymorphisms has gradually attracted attention.[15-17] In a study, Koponen et al found that the IL10 rs1800896 SNP is associated with asthma after severe lower respiratory tract infections in preschool children and infants.[17] In their study, Korppi et al also found that the SNP site (rs1800896) is associated with repeated wheezing in children.[15] Iron homeostasis in the human body can affect the immune state of the body. Under iron deficiency, children are at increased risk of allergic diseases such as eczema and asthma.[18] The membrane-bound serine protease encoded by the TMPRSS6 gene is a negative regulator of hepcidin production, which can reduce the expression level of hepcidin. A small-sample-sized study of infants suggested that children with the TMPRSS6 rs855791 TT genotype had an increased risk of CMA (OR=3.47, p=0.011).[19] The IL13 pathway plays an important role in food allergies.[20] The SNP site of the IL13 gene rs20541 (+2044G→A) is related to an increase in IL13 levels.[21] Zitnik et al found that the rs20541 polymorphism is related to CMA.[22] However, whether previously identified genetic variants are associated with CMA and the extent to which these SNPs contribute to the occurrence of CMA in Chinese children are unknown.

Therefore, the present study aimed to investigate the extent to which genetic variants (previously reported CMA-related SNPs: rs17616434, rs2069772, rs1800896, rs855791 and rs20541) contribute to the occurrence of CMA in Chinese children and to further explore whether a family history of allergy modifies such a genetic association with CMA among two hundred infants with CMA and 799 healthy controls aged 0–12 months from seven hospitals in China.

METHODS

Study participants

This is a prospective cohort study involving 999 participants aged 0–12 months from 7 hospitals in China who were born between March 1, 2020, and December 31, 2020. The participants were recruited from a population of infants attending growth and development clinics or health clinics without supplemental food. Regarding the study groups, the CMA group comprised infants diagnosed with milk protein allergy according to the MAP Guidelines for Milk Allergy in Primary Care,[23] and the control group comprised infants who had been exposed to milk protein and had no allergic symptoms (follow-up to 1 year of age when there were no symptoms; patients with symptoms during follow-up were confirmed or excluded by avoidance testing).

A total of 200 children diagnosed with CMA in pediatric outpatient clinics from March 2020 to December 2020 were selected as the CMA group. The diagnostic criteria were based on the MAP Guidelines for Milk Allergy in Primary Care.[23] Children with suspected clinical symptoms of CMA were diagnosed by positive food avoidance and provocation tests. At the same time, 799 healthy infants were selected as the healthy control group.

The study was approved by the Clinical Research Ethics Committee of People's Hospital of Peking University (protocol #2019PHB192-01). Written informed consent for both the study and genetic sampling was obtained from a parent or a legal guardian of all individual participants included in the study.

Epidemiologic and clinical information collection

Trained research nurses conducted face-to-face interviews using structured questionnaires, collecting information on parity, previous pregnancy, gestational age, date of birth, delivery mode, infant sex, birth weight, antenatal complications and parental age and atopy. Atopy was referred to as asthma, allergic rhinitis or atopic dermatitis.

Specimen collection: The sponge head in the saliva collector (children's version) was used to scrape the inner wall of the oral cavity 10 times, and then, it was placed in the preservation solution for storage.

Genotyping using an Illumina ASA gene chip: The ASA (Asian Screening Array) chip is Illumina's first whole-genome SNP chip designed based on 9000+ East Asian whole-genome sequencing data. The chip contains 700,000 markers. The chip was used to clarify the differences in gene polymorphisms between the CMPA group and the control group.

Calculation of the genetic risk score

The genetic risk score (GRS) is the sum of the number of risk alleles of five SNPs. The number of risk alleles for each individual was weighted according to the effect size of the SNP–trait associations. We categorized GRS into a low-genetic-risk group (≤5) and high-genetic-risk group (6 to 10).

Statistical analysis

Stata 15.1 was used for statistical analysis. Measurement data are expressed as the mean ± standard deviation (x±s), and count data are expressed as the frequency and per-

centage (%). Comparisons of the measurement data of two groups were analyzed by means of the t test, and comparisons of count data were analyzed by means of the chi-square test. Hardy–Weinberg genetic balance tests were used to determine whether samples were from the same population. Logistic regression was used to analyze the correlation between genes and clinical phenotypes. Logistic regression analysis was performed to compare the incidence of CMA. The following covariates were included in the statistical model: mode of delivery, sex, parental history of allergies, and gestational age. Additionally, the statistical model included the interactions between the SNPs and parental allergies. All values were 2-sided, and $p<0.05$ was considered statistically significant.

RESULTS

Demographic and clinical features

All five SNPs were in Hardy–Weinberg equilibrium ($p>0.05$). The main demographic and clinical characteristics of the study population are shown in Table 1. Among the 999 study subjects, 200 were in the CMA group, and the rest were in the control group. The differences in preterm birth rate, low-birth-weight rate, average birth weight and average gestational age of the two groups were statistically significant. Compared with the control group, the CMA group had a higher preterm birth rate (17.5% and 9.6%, respectively), a higher rate of low-birth-weight infants (11.0% and 6.6%, respectively), a lower average birth weight, and a lower average gestational age. In the CMA group, the proportions of first babies and first pregnancies were higher, and the differences were statistically significant. The difference in the ratio of birth season between the two groups was also statistically significant ($p<0.001$), with the rate of children born in spring in the CMA group being significantly higher than that in the control group (20% vs 9.4%). However, there were no significant differences in sex, average parental age, mother's method of conception, delivery method, or whether amniotic fluid was contaminated between the two groups ($p>0.05$). Regarding the feeding method, the rate of exclusive breastfeeding in the CMA group was lower than that in the control group (26.5% and 36.7%, respectively, $p=0.015$). In the comparison of the two groups with a family history of allergies, the proportion of parents with allergies in the CMA group

Table 1. Demographic and clinical characteristics of patients and control subjects

Parameters	Patients (n=200)	Control (n=799)	*p* value
Sex			
Male	113 (56.5%)	398 (49.8%)	0.091
Female	87 (43.5%)	401 (50.2%)	
Gestational age	38.6±2.29	38.9±1.84	0.026
Premature	35 (17.5%)	77 (9.6%)	0.002
Low-birth-weight infant	22 (11.0%)	53 (6.6%)	0.036
Birth weight (kg)	3.15±0.62	3.26±0.51	0.018
Parental age			
Maternal age	31.9±3.96	32.0±4.02	0.679
Paternal age	33.3±4.34	33.4±4.77	0.732
Fertilization method			
Natural conception	185 (92.5%)	748 (93.6%)	0.570
Assisted reproduction	15 (7.5%)	51 (6.4%)	
Delivery mode			
Vaginal	115 (57.5%)	449 (56.2%)	0.739
Cesarean section	85 (42.5%)	350 (43.8%)	
Amniotic fluid pollution	15 (7.5%)	47 (5.9%)	0.396
Previous pregnancy			
None	129 (64.5%)	439 (54.9%)	0.015
≥1	71 (35.5%)	360 (45.1)	
Parity			
None	160 (80%)	537 (67.2%)	<0.001
≥1	40 (20%)	262 (32.8)	
Season of birth			
Spring (Mar.–May)	40 (20.0%)	75 (9.4%)	<0.001
Summer (Jun.–Aug.)	86 (43.0%)	266 (33.3%)	
Autumn (Sep.–Nov.)	60 (30.0%)	348 (43.6%)	
Winter (Dec.–Feb.)	14 (7.0%)	110 (13.8%)	
Parents allergy			<0.001
Absent	76 (38.0%)	480 (60.1%)	
Paternal (P)/Maternal (M)	100 (50.0%)	271 (33.9%)	
P+M	24 (12.0%)	48 (6.0%)	
Symptoms			
Urticaria/atopic eczema	155 (77.5)	445 (55.7)	<0.001
Oral allergy syndrome	102 (51.0)	107 (13.4)	<0.001
Feeding patterns			
Breast feeding	53 (26.5%)	293 (36.7%)	0.015
Mixed feeding	135 (67.5%)	476 (59.6%)	
Artificial feeding	12 (6.0%)	30 (3.8%)	

was significantly higher(p<0.001).

Genotype and allele distribution of SNPs in the CMA group and control group

Five SNPs, namely, rs17616434, rs2069772, rs855791, rs1800896 and rs20541, were summarized. As shown in Table 2, SNP rs1800896 was solely associated with CMA. The T allele of rs1800896 (OR=1.60, p=0.042) was significantly higher in the CMA group than in the control group. After adjusting for environmental and perinatal factors (sex, gestational age, birth weight, feeding patterns, season of birth, delivery mode, previous pregnancy, parity and parental age), further study was conducted to determine whether there were differences in 5 single-nucleotide polymorphisms (SNPs) and GRSs between the CMA group and the control group. Logistic regression analysis found that rs1800896 was more significantly associated with the CMA (OR=1.68, p=0.033). Each 1 risk allele, and an increase in the GRS was suggestively associated with an 11% higher risk of CMA (1.11: 0.99-1.27, p=0.069). When the GRS was classified as low genetic risk (grs5 ≤5) or high genetic risk (grs5 >5) as a dichotomous variable, a 45% increased risk of CMA was found in the GRS high-risk group compared to the GRS low-risk group (1.45: 1.02–2.06, p=0.037), as shown in Table 3.

The impact of parental allergy on offspring and interaction analyses

Logistic regression analysis indicated that if one parent is allergic, the offspring's risk of CMA will increase 1.87 times (1.46–2.39, p<0.001), as shown in Table 3. For the interaction analyses, a product term of 5 SNPs and parental allergy (yes, no) was added into the logistic regression models, and we found that only rs1800896 interacted with parental allergy history (p=0.0097). To clarify the interaction between GRS and parental allergy, a further stratified analysis of the effect of parental allergy on offspring CMA was conducted based on different GRS groups (≤5, >5). As shown in Figure 1, in the GRS ≤5 group, the risk of offspring CMA increased 1.97 times (95% CI: 1.11–3.51) for each additional parent with allergies. In the GRS >5 group, the risk increased 2.63 times (95% CI: 1.78-3.90) for each additional parent with allergies. The effect of parental allergy on offspring CMA was greater in the high genetic risk group (GRS >5) than in the low genetic risk group (GRS ≤5). In addition, the participants were

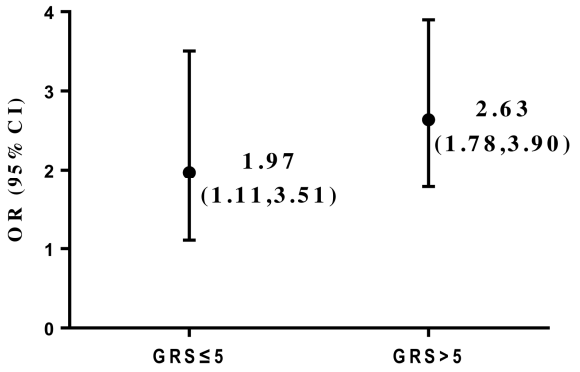

Figure 1. Parental allergy for CMA risk.

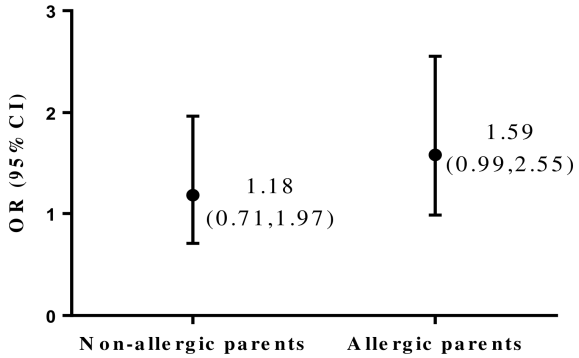

Figure 2. GRS for CMA risk.

stratified by whether the parents had allergies to study the influence of high genetic risk (GRS >5) and low genetic risk (GRS ≤5) on the occurrence of CMA in infants. The results show that the effect of high genetic risk of CMA in offspring was increased by 18% and 59% in the parental nonallergic group and parental allergic group, respectively (Figure 2). We found a positive additive interaction between the GRS of CMA and parental allergy. Figures 3(a) and 4(a) show the population distribution of the different GRSs in the CMA and control groups, respectively. The cumulative percentage for GRS ≤5 was 29.5% in the CMA group (Figure 3(b)) and 36.4% in the control group (Figure 4(b)). Figure 5 show a decision-making diagram for Chinese infants suspected with CM.

DISCUSSION

Our study found for the first time that the rs1800896 variant in the IL-10 gene is associated with CMA in Chinese children. A GRS constructed based on genetic variants that have been previously identified in the Western population was additively associated with the risk of CMA and had an interaction with a parental history of allergy, implying that genetic risk for CMA was exacerbated among those with a parental history. Our findings underscore the importance of early targeted therapeutic interventions for children with a parental history.

CMA is the most common allergic disease in infancy, accounting for 1/4 of childhood food allergies.[24] CMA can involve various systems throughout the body, with digestive system and skin symptoms, such as vomiting, reflux, diarrhea, blood in the stool, intestinal colic, eczema, and urticaria, as the main manifestations. Indeed, a growing body of literature has shown that FA significantly diminishes quality of life among affected patients and their caregivers,[25] who live in constant fear of accidental ingestion and potentially life-threatening reactions. To date, the mechanism of CMA remains unclear, but it is generally believed to be the result of a combination of environmental and genetic factors. Therefore, to explore the genetic characteristics of milk protein allergies, early diagnosis and treatment can allow infants to eliminate the intrusion of milk protein allergies early, reduce the impact of CMA on the growth and development of infants and young children, eliminate parental anxiety, reverse abnormalities in early immune status, avoid the develop-

Table 2. Comparison of SNPs between cow's milk allergy (CMA) and controls

SNP	Group	Risk allele	Frequency, n (%)	*p* value	OR (95% CI)	Genotype frequency AA/AB/BB, n (%)			‡*p* value
rs17616434						CC	CT	TT	0.903
	CMA	T	142 (35.5)	0.650	1.06 (0.838-1.33)	85 (42.5)	88 (44.0)	27 (13.5)	
	Control		548 (34.3)			351 (43.9)	348 (43.6)	100 (12.5)	
rs2069772						CC	CT	TT	0.684
	CMA	T	352 (88.0)	0.442	1.14 (0.816-1.59)	3 (1.50)	42 (21.0)	155 (77.5)	
	Control		1383 (86.8)			12 (1.50)	191 (23.9)	596 (74.6)	
rs855791						GG	AG	AA	0.493
	CMA	G	177 (44.3)	0.873	1.02 (0.817-1.70)	37 (18.5)	103 (51.5)	60 (30.0)	
	Control		700 (43.8)			163 (20.4)	374 (46.8)	262 (32.8)	
rs1800896						CC	CT	TT	0.107
	CMA	T	377 (94.3)	0.042	1.60 (1.01-2.52)	0 (0.00)	23 (11.5)	177 (88.5)	
	Control		1456 (91.1)			3 (0.40)	136 (17.0)	660 (82.6)	
rs20541						GG	AG	AA	0.305
	CMA	A	139 (34.8)	0.184	1.17 (0.93-1.47)	81 (40.5)	99 (49.5)	20 (10.0)	
	Control		500 (31.3)			372 (46.6)	354 (44.3)	73 (9.10)	

SNP: single-nucleotide polymorphism; OR: odds ratio; CI: confidence interval.
†*p* values for risk allele.
‡*p* values for genotype frequency.

Table 3. Genetic effects of SNP and parental allergies on cow milk allergies

Parameters	Model 1†		Model 2‡		Model 3§	
	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
rs17616434	1.05 (0.834–1.31)	0.692	1.05 (0.830–1.32)	0.706	1.05 (0.834–1.33)	0.664
rs2069772	1.13 (0.809–1.59)	0.470	1.15 (0.816–1.63)	0.420	1.16 (0.816–1.63)	0.417
rs855791	0.990 (0.796–1.23)	0.928	1.01 (0.809–1.26)	0.924	1.02 (0.810–1.27)	0.897
rs1800896	1.67 (1.050–2.69)	0.031	1.61 (1.01–2.59)	0.046	1.68 (1.04–2.71)	0.033
rs20541	1.19 (0.921–1.48)	0.201	1.15 (0.900–1.46)	0.267	1.16 (0.911–1.49)	0.225
grs5	1.11 (0.983–1.25)	0.092	1.11 (0.983–1.26)	0.091	1.11 (0.991–1.27)	0.069
GRS	1.40(0.996–1.96)	0.053	1.39 (0.983–1.97)	0.062	1.45 (1.02–2.06)	0.037
Parents allergy	1.94 (1.53–2.47)	<0.001	1.97 (1.55–2.50)	<0.001	1.87 (1.46–2.39)	<0.001

†Model 1 Adjusted for sex and gestational age.
‡Model 2 Adjusted for sex, gestational age, birth weight, feeding patterns and season of birth.
§Model 3 Adjusted for sex, gestational age, birth weight, feeding patterns, season of birth, delivery mode, previous pregnancy, parity and parental age.
¶grs5 =rs17616434+rs2069772+rs855791+rs1800896+rs20541.
††GRS was classified as low genetic risk (grs5 ≤5) or high genetic risk (grs5 >5), as a dichotomous variable.

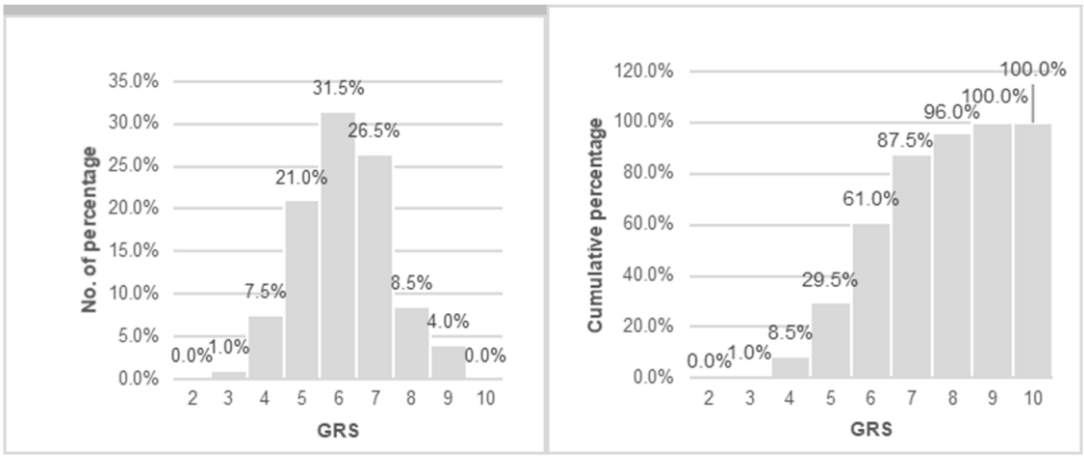

Figure 3. Distribution of GRS (a) population percentage and (b) population cumulative percentage in CMA group.

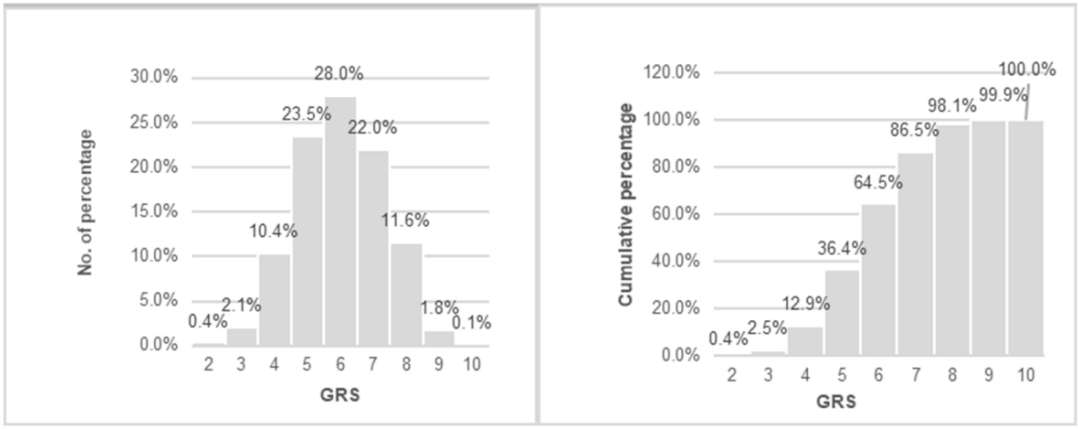

Figure 4. Distribution of GRS (a) population percentage and (b) population cumulative percentage in the control group.

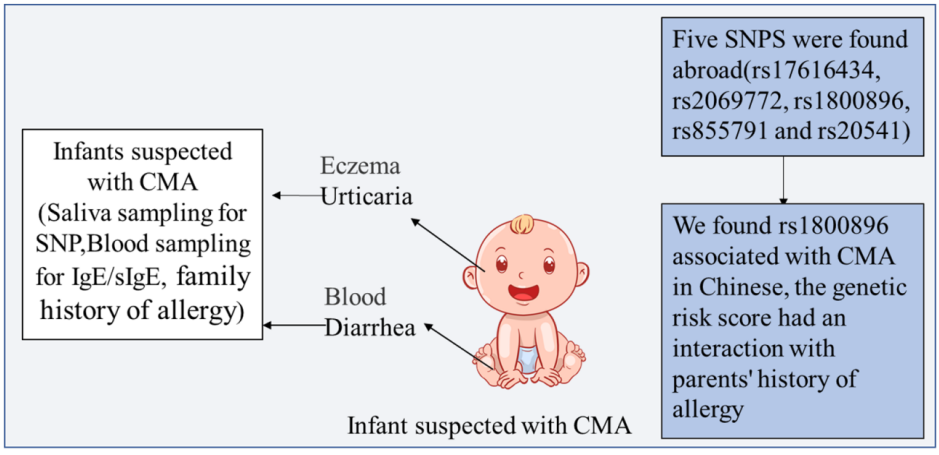

Figure 5. Decision-making diagram. Genetic susceptibility of cow's milk allergy.

ment of allergic processes, and lay the foundation for the health of infants and children.

The natural history of CMA is unique because in most patients, the symptoms resolve spontaneously. This complicates the evaluation of the prevalence of the disease. CMA usually occurs in the first 2 years of life and especially within the first year.[24,26,27] Therefore, this study selected infants aged from 0–12 months, and they were followed up to 12 months to determine whether they had allergies. The development of all food allergies is affected by genetics, environment, and genome-environmental interactions, including epigenetic effects.[27,28] Many risk factors for CMA have been identified or proposed to cause allergies or sensitization. The currently discovered CMA-related risk factors include sex, and males have a near twofold higher risk among children; however, the opposite is true in adulthood, with 80% of CMA patients being women.[3] We did not find sex-related differences in our previous study. Additionally, there are differences between races. McGowan et al compared the racial sensi-

tization rate of NHANES (National Health And Nutrition Examination Survey) participants (6 to 19 years old) and found that white participants had the lowest CMA allergy rate compared with black and Mexican-American participants.[29] It is generally believed that parental atopy significantly increases the risk of atopic diseases in the developing baby. It is one of the strongest risk factors, similar to other atopic diseases. Koplin et al[30] found that among one-year-old infants, compared with infants with no family history of allergic disease, infants who had one immediate family with an allergic disease had a 40% higher risk of allergies, and among infants with two relatives who had allergic disease, the risk of allergies rose to 80%. This study found that for every parent who has an allergy, the offspring's risk of allergies increases by approximately 1.9 times. In addition, preterm birth is a risk factor for CMA. Sardeecka et al reported that the risk of CMA in preterm newborns is increased,[31] which may be caused by the greater intestinal permeability of premature infants.[32] This study found that compared with the control group, the CMA group had a higher preterm birth rate (17.5% versus 9.6%), a higher rate of low-birth-weight infants (11.0% versus 6.6%), a lower average birth weight, and a lower average gestational age, which is consistent with the conclusions of previous reports. The mode of delivery may also affect the occurrence of CMA. The incidence of CMA among infants born by cesarean section may be higher because of the lack of the neonatal microbiota obtained by vaginal delivery, which in turn affects the infant's immune system.[32,33] However, no relationship between CMA and delivery type was observed in this study.

IL-10 is an immunomodulatory and anti-inflammatory cytokine that regulates the production of Th1 and Th2 cells and the secretion of cytokines during the immune response. Studies have found that the IL-10 gene polymorphism is associated with eczema, wheezing in infants and young children, childhood asthma, and the number of circulating eosinophils and IgE levels.[34,35] Rs1800896 is a single-nucleotide variant in the promoter region of the IL10 gene on chromosome 1.[36] Biologically relevant changes in promoter regions often alter the anchoring of different transcription factors, leading to differences in gene transcription. In their study of 125 children of Finnish descent, Holster et al found that the AA allele at locus rs1800896 was associated with an increased incidence of asthma, duration of asthma, and use of inhaled glucocorticoids compared to the AG or GG genotypes.[35] The meta-analysis published in 2014 included 4716 adults and children with asthma and 5093 controls and found that the IL-10 rs1800896 polymorphic locus was associated with asthma susceptibility in atopic children and adults.[37,38] A meta-analysis published in 2016 including 2494 asthmatic children and 2160 control children concluded that the IL-10 rs1800896 polymorphism may be a risk factor for childhood asthma.[39] The secretion of IL-10 is affected by genetic factors, and the level of IL-10 in the human body directly affects the susceptibility to and severity of diseases such as food allergies. A Brazilian study including 50 children with IgE-mediated CMA and 224 healthy controls found that the homozygous rate of the G allele at locus rs1800896 (IL10-1082A/G) was higher in the CMPA group than in the control group (19% versus 12%, p=0.027).[40] Our data show that the IL-10 rs1800896 gene polymorphism site is related to CMA and that there is an interaction with parental allergy history. Previous studies suggest that IL-10 rs1800896 is related to other late-onset allergic diseases (allergic rhinitis, asthma),[41,42] and Alduraywish found that food sensitization in the first two years of life increased the risk of subsequent asthma and allergic rhinitis.[43] These results indicate that CMA, allergic rhinitis and asthma have a common genetic cause, which also supports the "allergic march" hypothesis and the role of early-life food sensitization in the atopic march.

We found a positive additive interaction between the GRS of CMA and parental allergies. Genetic factors associated with a high risk of the GRS and parental allergies have a greater impact on offspring CMA. Additionally, the presence of parental allergies have a more pronounced effect of the GRS on CMA occurrence than lack of parental allergy. The cumulative percentage distribution in the low genetic risk (GRS <5) was higher in the control group than in the CMA group. These findings suggested that GRS has predictive significance for CMA and will inform the development of CMA risk prediction models in the future. In this study, no associations between other genetic models and CMA were found. This may be partly due to the short-term follow-up of the cohort and the assessment of allergic diseases based on the symptoms reported by the parents. Therefore, in the future, long-term follow-up and disease assessments through symptom evaluations and objective measurements (such as the skin prick test or serum allergen-specific IgE measurement) will be necessary.

This study has several limitations in this study. First, only five SNP loci (rs17616434, rs2069772, rs1800896, rs855791, and rs20541) were selected as candidate genes. There are few candidate genes, and these genes should include more allergy-related genes and SNPs. Second, genetic risk is classified according to the number of homozygous risk alleles. Failure to fully consider the single effect of each risk allele on the disease may weaken its effect. The GRS is an emerging method that integrates the weak effects of each risk allele and enables effective causal estimation of a large number of genetic variants. It has been widely used in genetic research of complex diseases. However, the GRS in this study had an interaction only with a parental history of allergy and did not obtain other positive results, which may be related to the small number of positive SNPs. Third, in this study, 999 children completed the 1-year follow-up and were evaluated for allergic diseases based on the symptoms reported by their parents. In future studies, larger samples, long-term follow-up, symptoms plus objective measurements, and repeated findings in other cohorts are needed.

Conclusion

In summary, our results show that the rs1800896 variant in the IL-10 gene is associated with CMA in Chinese children and that there is an interaction between this site and a parental allergy history on CMA, suggesting that genetic risk for CMA is exacerbated among those with a parental history. Our results underscore the importance of early targeted therapeutic intervention in children with a parental history. Moreover, our findings inform the future

development of CMA risk prediction models. In the future, long-term follow-up and functional and replication studies of this gene model are still needed.

AUTHIR DISCLOSURES

The authors declare no conflict of interest. This research was supported by the Foundation of 2018 Beijing Key Clinical Specialty Construction Project-Pediatrics (2199000726).

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Acknowledgements

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1. Zarrin R, Ibiebele TI, Marks GC. Development and validity assessment of a diet quality index for Australians. Asia Pac J Clin Nutr. 2013;22:177-87. doi: 10.6133/apjcn.2013.22.2.15.
2. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW et al. Obesity and the metabolic syndrome in children and adolescents. N Engl J Med. 2004;350:2362-74. doi: 10.1056/Nejmoa031049.

Book

3. Fildes VA. Breasts, bottles, and babies. A history of infant feeding. Edinburgh: Edinburgh University Press; 1986.

Chapter in a Book

4. Willett W. The use of biomarkers in nutritional epidemiology. In: Kok F, Veer P, editors. Biomarkers of dietary exposure. London: Smith-Gordon; 1991. pp. 9-14.

Internet linkage

5. Mahowald ML. Overview of the evaluation and management of gout and hyperuricemia. Rheumatology & Musculoskeletal Medicine for Primary Care, Gout. 2004/10/8 [cited 2005/5/12]; Available from: http://www.rheumatology.org/publications/primarycare/number4/hrh0021498.asp
6. Talukder A, Haselow NJ, Osei AK, Villate E, Reario D, Kroeun H et al. Homestead food production model contributes to improved household food security and nutrition status of young children and women in poor populations. Lessons learned from scaling-up programs in Asia (Bangladesh, Cambodia, Nepal and Philippines). 2000/2/17 [cited 2012/8/6]; Available from: http://factsreports.revues.org/404.

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Abbreviations

The following abbreviations are accepted without definition by APJCN

ANCOVA (analysis of covariance)
ANOVA (analysis of variance)
BMI (body mass index)
BMR (basal metabolic rate)
CHD (coronary heart disease)
CI (confidence interval)
CVD (cardiovascular disease)
df (degrees of freedom)
DHA (docosahexaenoic acid)
DNA (deoxyribonucleic acid)
DRIs (dietary reference intakes)
EDTA (ethylenediamine tetra-acetic acid)
ELISA (enzyme-linked immunosorbent assay)
EPA (eicosapentaenoic acid)
FAO (Food and Agriculture Organization) (except when used as an author)
FFQ (food-frequency questionnaire)
GC (gas chromatography)
Hb (haemoglobin)
HDL (high-density lipoprotein)
HIV (human immunodeficiency virus)
HPLC (high-performance liquid chromatography)
IHD (ischaemic heart disease)
LDL (low-density lipoprotein)
MRI (magnetic resonance imaging)
MUFA (monounsaturated fatty acids)
NS (not significant)
OR (odds ratio)
PCR (polymerase chain reaction)
PUFA (polyunsaturated fatty acids)
RDA (recommended dietary allowance)
RER (respiratory exchange ratio)
RIA (radioimmunoassay)
RMR (resting metabolic rate)
RNA, mRNA etc. ribonucleic acid, messenger RNA etc.
SFA (saturated fatty acids)
SNP (single nucleotide polymorphism)
UN (United Nations) (except when used as an author)
UNICEF (United Nations International Children's Emergency Fund)

UV (ultra violet)
VLDL (very-low-density lipoprotein)
WHO (World Health Organization) (except when used as an author)

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