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Letter to Editor

A Future Where Asia Works Together to Eradicate Malnutrition

8th Asian Congress of Dietitians (ACD2022)

The 8th Asian Congress of Dietitians and Nutritionists (ACD2022) was held at Pacifico Yokohama, Yokohama, Japan, from 19-21 August 2022. Due to the Corona pandemic, many national and international scientific meetings and congresses were conducted online; however, it was possible to hold ACD2022 face-to-face. The Asian Congress of Dietetic (ACD) is held every four years in one of the member countries of the Asian Federation of Dietetic Associations (AFDA), and this is the first time it was held in Japan. AFDA members include the Japan Dietetic Association, as well as the Dietetic Associations of Taiwan, Hong Kong, India, Indonesia, South Korea, Malaysia, the Philippines, Pakistan, Singapore, Thailand and Australia. The theme of ACD2022 was "Towards a sustainable healthy society for a bright Asian future: addressing confusing nutrition information to solve contemporary issues". The conference brought together 1,137 dietitians, nutritionists and other nutritionists from Japan and 17 other Asian countries to discuss nutrition challenges, as well as measures and policies in their respective countries through 23 lectures, symposia and workshops, and 272 poster presentations.

International Congress of Miracles begins

The ACD2022 opened with the soaring "I hereby declare the opening of ACD2022" by Ms Shihoko Suzuki, Vice-Chairperson of the Organizing Committee of the 8th Asian Congress of Dietitians (hereinafter referred to as the ACD2022 Organizing Committee).

At the opening ceremony, AFDA President Gordon Cheung, who was unable to visit Japan due to travel restrictions, sent a video message to the participants in the audience, encouraging them to enjoy the ACD2022 program and to develop friendships with other Asian dietitians. In his opening remarks as organiser, Professor Teiji Nakamura, Chair of the ACD2022 Organizing Committee, said, “Ladies and gentlemen, welcome to Japan. We have been able to hold this congress. Nutrition is not only for health, but is also related to education, economics, gender, etc., and supports the sustainable development goals (SDGs) that are moving towards their realization around the world. Solving the double burden of malnutrition in Asia will require developing and deploying nutrition leaders in every corner of every country. Let's have a lively discussion over the next three days with the aim of making the people of Asia healthier and happier," he said in his opening remarks.

Guests of honor at the opening ceremony included Tokue Seino, Director of the Nutrition Guidance Office, Health Division, Health Bureau, Ministry of Health, Labor and Welfare, on behalf of Katsunobu Kato, Minister of Health, Labour and Welfare; Yuji Kuroiwa, Governor of Kanagawa Prefecture; Hirotoshi Shiro, Deputy Mayor of Yokohama City; Okami Tsukahara, Vice President of the Japanese Society for Nutrition Improvement; and Yoshihiko Takigawa, President of the Japan National Dietetic Training Institute Association, who was present to celebrate the opening.

At the awards ceremony held in conjunction with the opening ceremony, the Dr Chwang Leh-Chii Asian Dietetics Award was presented to Shigeru Yamamoto of the Japan Dietetic Association and Chanida Pachotikarn of the Thai Dietetic Association. The Dr Ching-Hwa Chiu Asian Outstanding Young Dietitian Award for young dietitians under the age of 45 was also presented to Ms Frankie Pui-Lam Siu of the Hong Kong Dietetic Society. Frankie took to the stage online and said, "My dream is to connect with and learn from other dietitians in Asia, and to put that back into advising patients. I believe that we as dietitians can create great power by outputting our knowledge and passion".

The impact of Japan Nutrition

In a special lecture entitled 'Japan Nutrition', Chairman Nakamura Teiji presented Japan's nutrition policy to date and the activities of dietitians. AFDA Honorary President Chwang Leh-Chii, who chaired the session, expressed his surprise when he explained that in 1926 there were only 13 dietitians in Japan, whereas today there are more than one million dietitians and nutritionists. He praised the fact that there are one million dietitians in the country, saying that it is first of all a great achievement.

Chairperson Nakamura emphasized that Japan has continued to improve nutrition in a comprehensive manner based on nutrition education for the people by converting the food policy of the United States into a nutrition policy in Japan during the period of food shortages after World War II, implementing proper supply and distribution of food, and placing nutrition specialists in various institutions in Japan, including government, healthcare, schools, welfare facilities, companies, Self-Defense Forces, prisons and universities, etc. The Japan Dietetic Association (JDA) has been working to improve the nutritional quality of the Japanese people, and has been promoting the existence of Japan as a country with a long life expectancy. He added: "I am convinced that nutrition in Japan is sustainable, leaving no one behind and making healthy longevity possible. I believe that everyone in Asia agrees with me. Let us work more closely and amicably and contribute to Asia," he summarized, to which the audience agreed with loud applause. The speech was concluded by former Speaker of the House of Councilors, Akiko Santo, who, along with her congratulatory remarks, stated that she would do all she could to back the cause of improved nutrition in Asia.

Mutual learning and discussion of Asian “Dietetics”

The three-day programmer consisted of one special lecture, two keynote lectures, four educational lectures, nine symposia and seven workshops with a total of 23 contents, covering a wide range of topics from diabetes, obesity, infectious diseases, frailty and sarcopenia in clinical and public nutrition to policies and systems such as disaster support, food service systems and nutritionist training, as well as a workshop where young nutritionists from different countries discussed their vision for future nutritionists.

One of the programs, Workshop 2: Dietitian Activities in Obesity Control, featured speakers from Indonesia, Thailand and Japan, each of whom explained their country's policies and current situation, followed by a discussion involving audience participants. Miranti G. Sumapradja, an Indonesian dietitian, citing the Indonesian dietetic habits of heavy use of salt, sugar and coconut milk in cooking and a preference for fried foods, and the country's lack of parks and places for outdoor exercise, said, "There are about 50,000 professionals involved in nutrition, but dietitians are only 10-15% of them exist, which is very small for a population of 170 million people. The government is trying to train more dietitians, and if there was one dietitian per 10,000 people, the current obesity rate of 21.8% could be reduced even further". He added that a cultural approach is also needed to combat obesity, noting that "in Indonesia, there is a practice where chubby infants are seen as being “well looked after by their mothers". Samitti Chotsriluecha, a Thai dietitian, also commented, “I am impressed that Japanese people, young and old, walk a lot. I think the fact that sweet food is also favored in Thailand and that there is little physical exercise is a factor in the increase in overweight, especially among middle-aged women", describing the current situation.

While the situation in various Asian countries was presented in various sessions, Chairman Nakamura spoke in Workshop 3: "Towards realising the commitments of the “Tokyo Nutrition Summit 2021”, saying that “the word 'Dietetics” in the Asian Congress of Dietetics is difficult to translate into Japanese, as it includes not only nutrition, but also culture, economy and preferences. The time has come to disseminate 'Dietetics' from Asia, without relying on standards and guidelines from the West"

Poster presentations and exhibitions to deepen friendships

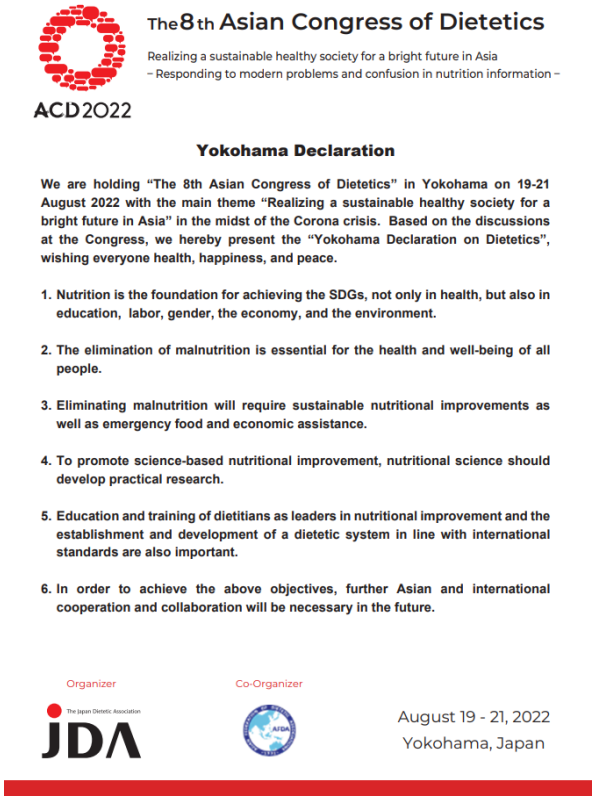
In the exhibition hall, 272 posters were presented, with presenters opening their laptops on the round tables provided and presenting their findings to the assembled participants. The posters were posted online allowing participants to view them from a distance on their own devices and communicate with the presenters via a chat room. Also in the exhibition hall, Yakult Honsha Co Ltd, Ajinomoto Co Ltd, Meiji Co Ltd, California Walnut Association, "Rice Flour Gentle Swallowing Food" Consortium, Friesland Campina Institute, Hasegawa Chemical Industry Co Ltd, Kikkoman Foods Co Ltd, Nipun Co. Japan Sports Nutrition Association, and Nutrition Support Network LLC exhibited and promoted their initiatives to visitors. In addition, a variety of other 'hospitality' activities were offered, including a booth where visitors could easily experience the tea ceremony, a booth where they could relax with yoga, a photo booth where commemorative photos could be taken, a rest area and a drinks corner.

Signing of the Yokohama Declaration

After three days of lively discussions and deepening their friendship, the dietitians from the different countries came to the closing ceremony on the 21st (Sun) with a sense of farewell. In his closing remarks, Chairman Teiji Nakamura thanked all those involved and continued as follows. 'One thing became clear during our discussions at ACD 2022. Of course economic and food aid is necessary to eliminate malnutrition in the world, but at the same time we need to educate and train professionals. There is a need to provide solid education for the profession and to increase public awareness of dietitians. Asia has the highest population growth and economic growth rates in the world. This has led to significant changes in dietary habits. It is necessary to share information and work together to solve problems across Asia. It is an achievement to have spent three days discussing this with each of you. “Making people healthier and happier through the power of nutrition” - see you again in India in four years' time.’

After that, Dr Yasuhiro Kido, Chair of the Executive Committee of the ACD2022 Organizing Committee, gave a summary of the number of participants and other details of ACD2022 and presented the Poster Awards. 13 presenters were recognized and Mr. Ying Qian Ong of the Malaysian Dietetic Association spoke on behalf of the winners and said, "Many people helped to make this research that has been recognized. I would like to send this award to everyone who helped us". The young award winners were all smiling and happy as they were presented with their awards on stage, symbolizing the bright future of nutritionists in Asia.

Furthermore, as an outcome of the congress, the ' Yokohama Declaration' was announced and signed on stage by the representatives of each country. Finally, a video documenting the three days and a promotional video of the next host country, India, were shown. ACD2022 was then declared closed by Ms Toshiko Saito, Vice-Chairperson of the ACD2022 Organizing Committee. As stated in the ' Yokohama Declaration', nutrition is fundamental to achieving the SDGs. Eliminating malnutrition is also essential for the health and well-being of all. At the AFDA General Assembly held during the ACD 2022 session, it was decided that Japan will be the chair country of AFDA from now until 2026 and that Dr. Teiji Nakamura President of the Japan Dietetic Association, will assume the position of AFDA President. As committed to at the Tokyo Nutrition Summit 2021, the Japan Dietetic Association will promote the "Training and deployment of food and nutrition professionals to build a sustainable nutrition improvement infrastructure", particularly in Asia. The mutual understanding and friendship with other countries fostered by ACD 2022 will be used as a driving force to develop new initiatives.



Letter to editor

Becoming A Dietitian in Asian Countries: Educational Aspects from Young Dietitians' Perspective

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Dear Editor:

Dietitians, who are referred to as Registered Dietitians (RD) or Registered Nutritionist Dietitians (RND) in some countries, are qualified regulated health professionals dealing with human nutrition and dietetics issues faced at any life stage. They assess, diagnose and treat dietary and nutritional problems at an individual and wider public health level (BDA Curriculum 2020) (1). They also apply the science of food and nutrition to promote health, prevent and treat disease to optimize the health of individuals, groups, communities, and populations (International Confederation of Dietetic Associations (ICDA, 2014) (2). The subjects of dietitians' practice range from hospital patients, school children, elderly in long-term care facilities, or even professional athletes. Thus, dietitians have to complete an education curriculum and satisfy the requirements to prove they are capable of accomplishing the professional tasks as regulated by national authorities.

From August 19th to 21st, 2022, young dietitians from six Asian countries (Japan, Taiwan, Thailand, Philippines, Indonesia, and Vietnam) gathered at The 8th Asian Congress of Dietetics (ACD 2022) in Yokohama, Japan. For the first time, we shared our ideas about dietitians' education and practices in our countries. Given limited opportunities to gather and share our thoughts, everyone was eager to bring up a broad range of topics, such as the legal system related to dietitians, strategies to fight obesity, and malnutrition treatments in clinical settings. We have been meeting online twice a month from April to August, preparing the topic we wanted to share with Asian dietitians during ACD 2022. An important topic during our meetings was the education programs compulsory to be dietitians in each country. Through the online meetings and congress, we sought how Asian countries could build a mutual understanding as a foundation to develop a better education program for dietitians in the future. Here is a glance at our perspective.

Practice hours and internship

Accreditation standards for dietetics education programs across the world, require curriculum to meet the foundational knowledge, skills and attributes needed for dietetic practice. The curriculum commonly incorporates a range of credits acquired by attending lectures, experimental/practical courses (including simulation, case studies, and role-playing) at colleges or universities, as well as taking professional practice (practical training/internships) programs in healthcare or service facilities. All countries grant pupils diplomas, bachelor's, or master's degrees by fulfillment of the education curriculum, which might be followed by additional professional practice, passing national examinations, and official administrations before they are granted their dietitian license.

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A consensus on the core concepts for the dietetics discipline and dietetic competency standards among the countries has been acknowledged. In addition to on-campus courses, professional practice in healthcare facilities, public health centers, or food service facilities is an essential part of a dietitian education program to equip prospective dietitians with comprehensive skills for their professional tasks in the future. Naturally, the number of training hours in real settings needs an extended consideration since the skills acquired from those practices are proportional to the amount of practice taken. According to International Standard for Education of Dietitians-Nutritionists by ICDA, the minimum level of education of a dietitian-nutritionist is a bachelor's degree and a period of supervised professional practice of at least 500 hours and meets the international competency standards for dietitians-nutritionists (2). In western countries like the British Dietetic Association, students are expected to undertake not less than 1000 hours of practice-based learning with not less than 350 hours in the clinical setting and not more than 500 hours in one setting (1).

Despite international standardization attempts, qualifications and education standards to become a dietitian differ in each country. We have realized that these standards may differ due to the specific conditions of the dietetic organizations in the context of the country's education and health systems and the cultural, social, and historical experiences of the country. The varied qualifications and education standards in Asian countries are summarized in table 1.

Among all Asian countries, the Philippines has the highest education units in their Bachelor's program in Nutrition and Dietetics as they follow the United States of America's standard. For the Bachelor's program in the Philippines, a total of 165 units which is a combination of general education and professional courses should be completed by the student to be able to graduate in the program. This program includes 3-year didactic teaching and 1-year practical training or practicum in hospitals, food service, and the community. Schools or universities

offering BSND program or equivalent is flexible when it comes to their curriculum however when it comes to

Table 1: A summary of education programs, practice regimes, and Registered Dietician (RD) eligibility among some Asian countries

Countries	Education programs	Credits + Practice hours (At university)	Practice hours (Outside university)	Internship (After graduation)	RD eligibility	Renewal RD qualification
Japan	Diploma, BSc, MSc, PhD	810hr (18cr.)	180hr (4cr.)	N/A	Accredited course + Practice + Exam	N/A
Taiwan	Diploma and BSc	20 Credits	500hr (7cr.)	2 years (hospital)	Accredited course + practice hours + Exam	120hr. credits every 6 years
Thailand	BSc and MSc	BSc (>135cr.) 525hr	900hr	1-2 years (if not finish 900hr)	Accredited course + Internship + Exam	N/A
Philippines	BSND, MSc, PhD	BSc (136 units lecture) + (40 units lab)	1200hr/ 1 year (24 units)	N/A	Accredited course + Practice hours + Exam Mutual Reciprocity in US as RD: RND + Exam	CPD units (45), Member of NDAP
Indonesia	BSc	272-408hr (6-9cr.)	317-453hr (7-10cr.)	1723hr (38cr.)	Accredited course + Internship + Exam	Every 5 years
Vietnam	BSc and MSc	Credits: 35 450hr	At hospital: 254hr	N/A	N/A	N/A

BSc: Bachelor of Science; CPD: Continuing Professional Development; MSc: Master of Science; NDAP: Nutritionist-Dietitians' Association of the Philippines; RD: Registered Dietician; RND: Registered Nutritionist Dietitian.

professional courses, they should implement as prescribed in the program set by the Commission on Higher Education.

In Figure 1, the practice hour is divided into on-campus training (laboratory or alternate supervised experiences such as simulation, case studies, and role-playing inside the university) and at training sites (in professional work settings such as hospitals, catering companies, community centers…). Compared to the international standard, Taiwan and Thailand have met the

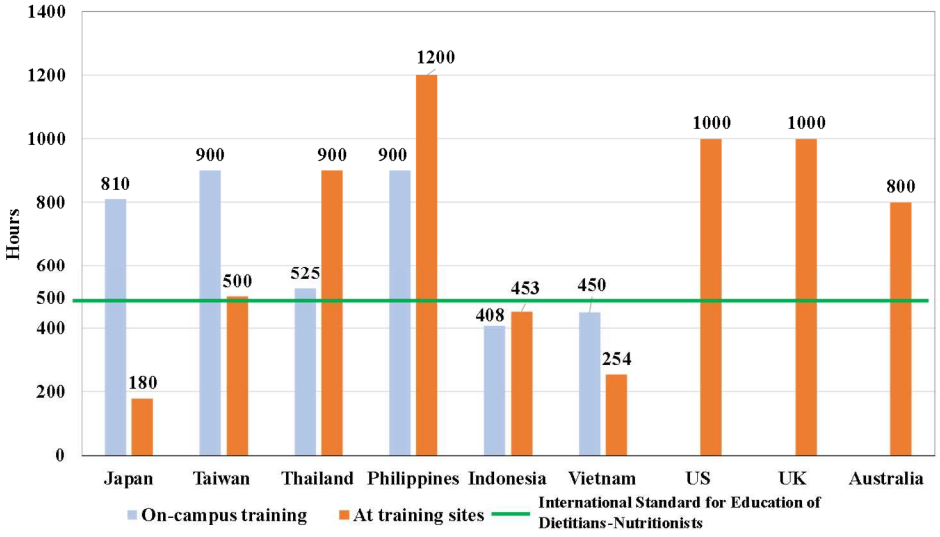

Figure 1. Practice hours during the Bachelor of Dietician/Registered Dietician program in some Asian countries and others

standard with both the practice hours on-campus and at the training sites ranging from 500 hours to 900 hours. The Philippines follows the standards of the Accreditation Council for Education in Nutrition and Dietetics (ACEND - Academy of Nutrition and Dietetics) which is 1200 hours for the practicum program (3). On the contrary, Japan spends more time on on-campus training and only 180 hours practicing in hospitals or companies. Both Indonesia and Vietnam have practice hours lower than 500 hours in either category. In comparison to western countries like the US, UK, and Australia where dieticians are required to have at least 800-1000 hours of on-site training, most Asian dietetics programs seem to spend less time on it (1, 3-4).

Not only the duration of training but different countries also have different standards of training protocols. In Thailand, Vietnam, and Indonesia, their on-campus training may focus only on basic science laboratory training due to the limitations of university facilities. It found some difficulties to develop and set up the simulator lab like the training sites. Therefore, they mostly spent more practice hours in the real training sites. However, in Japan, Taiwan, and the Philippines, with well-established dietetic-related laboratories (e.g. food service laboratory, counseling clinics, and patient simulators), they have more opportunities to practice both on-campus and at the hospital training sites.

With a shorter practice time, some countries like Taiwan and Indonesia require 2 years or nearly 2000 hours of after-graduation professional practice or (below mentioned as internships to contrast it with the professional practice during diplomas or undergraduate programs) in the hospital if one would like to work in the clinical setting. While the internship can help dieticians achieve competency and continue their learning, it can also affect their livelihood as some internships are unpaid. On the day of the workshop, we had the chance to discuss whether to spend more time on practice in the university or reduce the burden of practice in undergraduate training and complete the training from the internship. In 2003, the Institute of Medicine (IOM) proposed that healthcare professionals could no longer assume that they can practice effectively using the training received in their undergraduate education, as human memory could no longer continue to retain the whole knowledge-base on effective healthcare and was becoming increasingly unreliable in keeping pace with the ever-expanding healthcare research (5). The IOM recommended that for health professionals to meet the needs of the 21st-century health setting education reform was needed, however, they recognized an "overly crowded curricula" as a challenge. Therefore with these in consideration, we would like to suggest a manageable number of practice hours around 800-900 hours in professional work settings along with a one-year internship after graduation. With this, dieticians will be able to assure the international competency standard and evolve to be adaptable, work in new emerging areas of practice, and demonstrate critical thinking skills to deal with increasing medical complexity and an aging population.

Registered Dietician (RD)/ Registered Nutritionist Dietitian (RND)

Regardless of the difference in curricula or requirements of internships, almost every country, except Vietnam as they are still establishing its dietetics system, demands its dietitian candidates take and pass the National Board exam to be a Registered Dietician (RD)/Registered Nutritionist Dietitian (RND). The exam in most countries includes assessing the 3 main parts: basic nutrition knowledge, medical nutrition therapy, food service and management, and other supporting roles; community nutrition; and research methodology. After the board examination, the RD/RND can continue education through a Master's degree or may upskill in their chosen field of expertise (Renal Dietitian, Diabetes Dietitian, Pediatrics Dietitian, Sports Nutritionist, etc.). Registered Nutritionist-Dietitian in the Philippines may also take the Registered Dietitian examination in the US and may practice there once passed. This is from the Mutual Reciprocity of both countries.

Renewal of license was also discussed in the workshop. Japan and Thailand have no renewal of licenses while the Philippines, Indonesia, and Taiwan have specific guidelines when it comes to the renewal of licenses (Table 1). However, since the scope of practice and work of dietitians is continuously changing, developing, and diverse, it is necessary to encourage all national RD licenses to have a process of renewal. The renewal process may help dietitians update their knowledge regularly. It can increase continuing professional development skills and lifelong learning skills in parallel with practicing competencies.

Conclusion

Lastly, it was a great opportunity for the group of young Asian dietitians to join the workshop together in ACD 2022 to discuss each country's perspective. One of the selected topics, the dietetic legal system focusing on the eligibility to become a dietitian has been elaborated on as mentioned in this article. Although most countries have their own scopes of practice and national standards, they seem to have a similar core practice concurrent with the US, and ICDA standards. There would be more impact on our Asian countries if we could bring the collaborations to establish the standards of the dietitian system. This may go further with beneficial outcomes with the dietetic student exchange programs, working across Asian countries, and fruitful collaboration in the near future. These attempts are surely in line with the "Yokohama Declaration on Dietetics" which has been signed by the participating countries by the end of ACD 2022, declaring in points 5 and 6 as follows.

"Education and training of dietitians as leaders in the nutritional improvement and the establishment and development of a dietetic system in line with international standards are also important."

"In order to achieve the above objectives, further Asian and international cooperation and collaboration will be necessary in the future."

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Review

The Best Compositions of Late Evening Snack for Liver Cirrhosis Patients Using Systematic Review and Network Meta-Analysis

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ABSTRACT

Background and purpose. Liver Cirrhosis (LC) is ranked the 11th in the world and the 10th in Vietnam among common causes of death. Malnutrition frequently imposes a burden on patients with LC, and it is an independent predictor of lower survival. However, in Vietnam, the nutrition management in LC patients is insufficient. Moreover, glycogen storage is decreased in LC patients so if they do not have a late evening snack (LES), they will feel a lack of energy when they wake up the next day. However, a nutrient composition for LES has not been unified yet, there is a lack of evidence in comparing LES compositions with each other. ***Method.*** Network meta-analysis (NMA) was conducted to compare the effectiveness of different compositions of LES from direct and indirect evidence. The evidence was the published articles with intervention trials using different types of LES. All the papers searched from 5 databases (PubMed, Cochrane library, Google Scholar, ScienceDirect, Medical Online) until March 1st, 2022 that met the inclusion criteria were selected. The outcome parameters were albumin, pre-albumin, nitrogen balance, respiratory quotient, AST, ALT, total bilirubin, ammonia, cholinesterase, and BCAA/Tyr. ***Results***. After screening and full-text assessing 270 papers, 15 final papers with 4 types of LES (high CHO, Protein-CHO, BCAA-CHO, and coconut milk-CHO) were included in the meta-analysis. One of the most important parameters is albumin, an effective indicator in the management of cirrhosis and its complications. And according to the meta-analysis, Protein-CHO has the highest probability of improving it, followed by high CHO, coconut milk-CHO, and then BCAA-CHO. Other parameters (such as Pre-Alb, nitrogen balance, ALT, AST…) also indicated the same results. On the other hand, BCAA-CHO was not effective on most of the parameters but only on BCAA/Tyr. There was no direct comparison between BCAA and protein. ***Conclusion***. The recommendable LES compositions should provide at least 200kcal with 50g of carbohydrate. About the nitrogen source, protein is maybe more effective than BCAA in terms of improving protein synthesis and energy metabolism as well as liver parenchyma damage. However, since there is no direct comparison between protein and BCAA as LES, a future study is needed to confirm this finding.

Key Words*:* Cirrhosis, late evening snack, protein, network meta-analysis.

INTRODUCTION

Liver cirrhosis (LC) is one of the leading causes of mortality worldwide, it was the 11th most common cause of death each year in the world with 2.1% of total deaths (1). According to Institute for Health Metrics and Evaluation (IHME), in 2019, LC ranked 7th among the top 10 most common causes of death in Vietnam with a 47.3% increment from 2009 to 2019 (2). The etiologies of LC are most commonly alcohol, hepatitis B, hepatitis C, and non-alcoholic fatty liver disease or sometimes autoimmune hepatitis. Alcoholic liver disease and hepatitis B are the most common causes in most parts of Asia (3). Cirrhosis is defined as the histological development of regenerative nodules surrounded by fibrous bands in response to chronic liver injury, which leads to portal hypertension and end-stage liver disease. In the asymptomatic phase of the disease, usually referred to as compensated cirrhosis, patients may have a good quality of life, and the disease may progress undetected for several years. The decompensation phase is regularly marked by ascites, gastrointestinal bleeding due to esophageal varices, hepatic encephalopathy (HE), and jaundice (4).

Malnutrition is frequently a burden in patients with LC; it is usually related to the clinical stage of chronic liver disease, increasing from 20% in patients with well-compensated disease to more than 60% in patients with advanced cirrhosis (5). Malnutrition and muscle mass loss (sarcopenia) are associated with a higher rate of complications such as susceptibility to infections, hepatic encephalopathy (HE), and ascites, as well as being independent predictors of lower survival in cirrhosis and in patients undergoing liver transplantation (6). Various mechanisms are considered to contribute to malnutrition in cirrhosis such as poor oral intake, increased intestinal protein loss, decrease protein synthesis, disturbances in substrate utilization, hyper-metabolism, and malabsorption (7). According to Trang Thu Nguyen et al. study in 2020, the prevalence of malnutrition in LC patients was 60% by subjective global assessment (SGA), an international questionnaire for nutrition assessment (8). According to ESPEN's clinical nutrition practice guideline for LC patients in 2019, cirrhotic patients should ingest an increased amount of energy (30-35 kcal/kg/d of energy and 1.2-1.5 g/kg/d of protein) (5).

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Nonetheless, in Vietnam's clinical setting, a restricted protein diet is still recommended for LC patients (9). As a result, the energy intake and protein intake of Vietnamese LC patients were lower than their recommendation, 1445.9 ± 727.7kcal/day and 1.0 ± 0.5g/IBWkg/day, respectively (8).

In addition, several guidelines have recommended short periods of starvation with 4 - 6 meals a day, especially a late evening snack (LES) is highly recommended as LC is characterized by a state of accelerated starvation, with an early shift from glucose to lipid and amino acids utilization for energy during the postabsorptive state (fasting), which can also lead to a decrement in respiratory quotients of LC patients (10). Some systematic reviews and meta-analyses have proved that LES intervention helped to improve liver biochemical parameters for albumin, ammonia, respiratory quotients, and liver enzymes including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (11, 12). Moreover, increasing the number of meals can also help to increase the amount of dietary intake in LC patients. However, 77.5% of Vietnamese LC patients did not have LES and only 22.5% had the snack but the compositions of the snacks were inconsistent (8).

Having said that, it is also necessary to find the most suitable compositions of LES for Vietnamese patients from taste-wise to economically. In a systematic review, Tsien et al. recommended a 200kcal with 50g carbohydrate (CHO) late evening snack to minimize gluconeogenesis and preserve muscle mass (12). However, there has been a trend of using branched-chain amino acids (BCAA) in LES and some studies suggested it was more effective than CHO-LES (13-14). In addition, there were a number of studies that used a snack including both protein and carbohydrates, which showed improvement in LC patients as well (15-17). It seems that there have been a variety of compositions of LES used across different studies and to date, there is no trial comparing the potential effects of different LES in cirrhotic patients, thus defining effective compositions among CHO, BCAA, protein, no LES…are necessary.

To attain a better understanding on this issue, the available published trials are estimated by updated Bayesian network meta-analysis in order to investigate the efficacy of improving various parameters of liver cirrhosis patients between the use of different LES compositions. The results are expected to provide a reference for clinical practice.

METHODS

The study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) extension statement for reporting network meta-analyses of health care interventions (18).

Eligibility criteria. The eligibility criteria are detailed below following the participants, intervention, controls, outcomes, and study design (PICOS) framework: (i) Participants: adults (age >18 years) with cirrhosis, male and female; (ii) Interventions: any types of late evening snack (LES) that provides about 200kcal; (iii) Comparisons: studies that compared 1 or more LES compositions; (iv) Outcomes: At least one of these parameters: albumin (Alb), pre-albumin, nitrogen balance (NB), respiratory quotient (npRQ), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), ammonia (NH3), cholinesterase (Che), and BCAA/Tyr (BTR); (v) Studies lasting ≥ 1week. All the published articles with original data (except conference abstract and case report) in any language up to 01 March 2022 met the listed criteria were included.

Studies including patients in acute phase or hepatic encephalopathy and/or a specific health condition such as diabetes, stroke, chronic obstructive pulmonary disease, chronic kidney disease, other critical illnesses, and recent transplants were excluded. Studies using other nutritional interventions other than LES or patients who took other nutritional supplements before the study were also excluded.

Search strategy. The following five electronic databases were searched until March 2022: (i) PubMed, (ii) Cochrane Library, (iii) Google Scholar, (iv) ScienceDirect, and (v) Medical Online using the following terms: ("Liver Cirrhos*"[Mesh] OR "Cirrhos*" OR "Hepatic cirrhos*" OR "Liver Fibros*" OR "Hepatic Fibros*" OR "end-stage liver disease" OR "Advanced liver disease") AND ("Snacks"[Mesh] OR "Late evening snack" OR "nocturnal nutritional supplementation" OR "nocturnal snack" OR "evening snack" OR "nocturnal meal" OR "bedtime snack" OR "late night snack" OR "midnight snack" OR "late evening meal"). In addition to electronic database searches, cross-referencing was conducted by examining the reference lists of previous review articles as well as each included study for potential articles that met the inclusion criteria.

Study selection. All retrieved articles were combined in Endnote20 and Microsoft Excel (V.2016; Microsoft Corporation; 2016) to remove duplicates. After screening the titles and abstracts according to the prespecified criteria, the full texts of articles that potentially met the eligibility criteria were reviewed. Reasons for excluded studies were recorded using the following categories: (i) inappropriate population, (ii) inappropriate intervention, (iii) inappropriate comparison(s), (iv) inappropriate outcome(s), (v) inappropriate study design and (vi) other.

Data extraction. Microsoft Excel (V.2016; Microsoft Corporation; 2016) was used to develop comprehensive electronic codebooks. The major categories of variables coded included (i) study characteristics (author, journal, year of publication, design, etc.), (ii) participant characteristics (age, gender, liver cirrhosis etiology, LC severity, etc.), (iii) intervention characteristics (type, length, frequency, nutrients compositions, duration, etc.) and (iv) data for outcomes (sample sizes, baseline and post LES means and SD, etc.). When relevant information on design or outcomes was unclear, or when some needed data was unavailable directly from the study, the original authors sought eligible data by email.

Risk of bias and quality assessment. The Revised Cochrane risk-of-bias tool for randomized trials (RoB 2) [19] was used to assess the methodological quality of the randomized controlled trials (RCTs), and the Quality Assessment Tool for Quantitative Studies (19) assessed the quality of controlled pre-post studies and nonrandomized experimental studies (non-RCTs). In both quality assessment tools, each domain will be

considered as strong, moderate, or weak and studies will be classified as high, moderate, and low risk of bias.

Statistical analysis. Network meta-analysis (NMA) was conducted to estimate the effectiveness of different types of LES compositions on various parameters. The results of the comparative effects are presented as the mean differences (MDs) or standardized mean differences (SMDs) and 95% confidence intervals (CIs). We also estimated the ranking probabilities of the intervention effect using the surface under the cumulative ranking curve (SUCRA) (20). The larger the SUCRA value, the better the ranking of the intervention effect. The consistency between the direct and indirect evidence was evaluated using inconsistency tests to assess the validity of the transitivity assumption. Publication biases or small sample effects were examined using a comparison-adjusted funnel plot.

In addition, a pairwise meta-analysis using the random-effects model was performed when the data was not sufficient for a NMA (Pre-albumin and Nitrogen balance), and the I2 statistic and p values were calculated as a measure of the statistical heterogeneity (21), with I2 ≥ 50% indicating substantial heterogeneity.

Stata version 17 was used to conduct the analyses. The "metan" package was used for the pairwise meta-analysis, and the "network" package was used to conduct the NMA. Statistical significance was set as a P value < 0.05 in all analyses.

RESULTS

Study selection

A total of 267 articles were retrieved by following the pre-designed literature retrieval strategy. By further searching the references included in the articles, 3 additional articles were obtained. After reading the titles and abstracts, 43 studies were selected for further review. Finally, 15 studies met the inclusion criteria (13-17, 22-31). The detailed process of the search strategy is described in Figure 1.

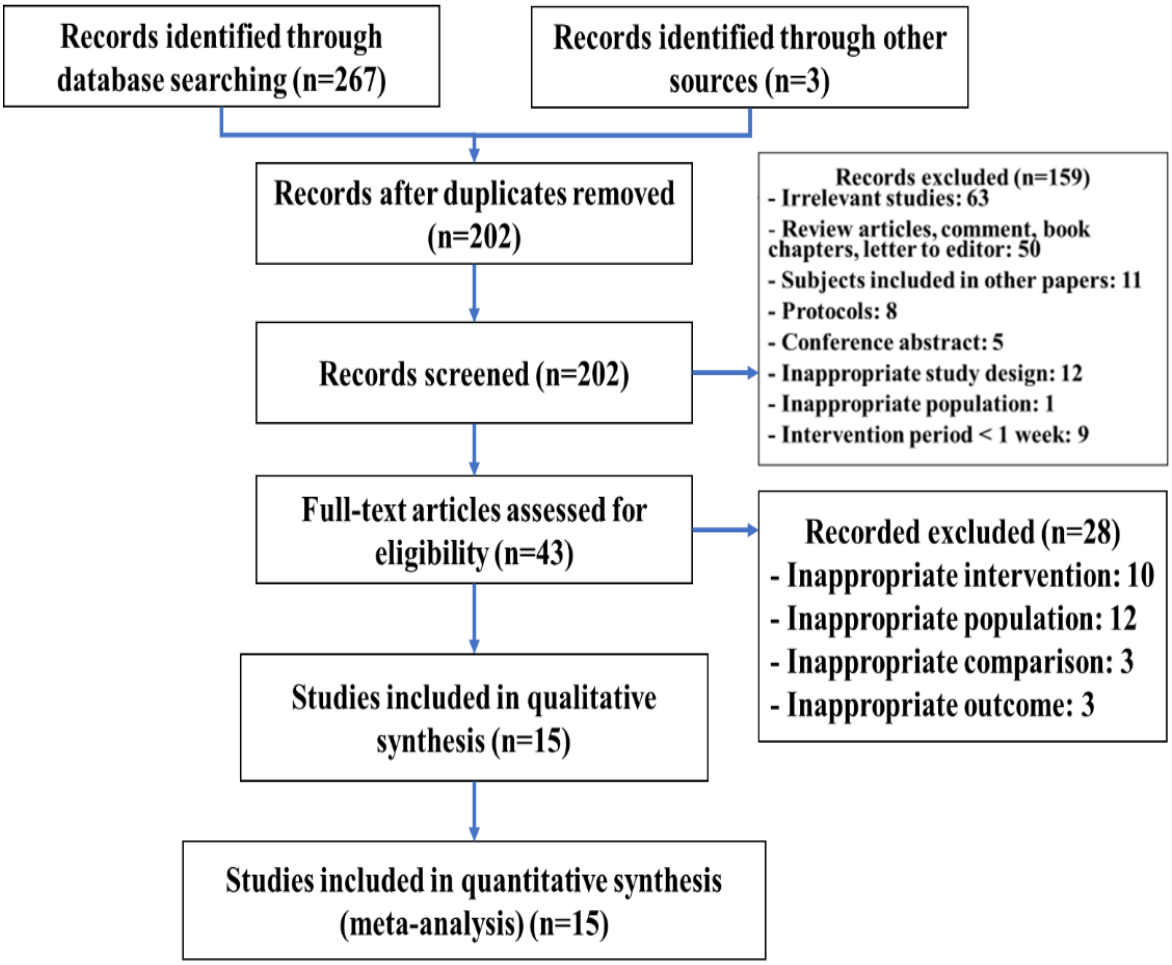

Figure 1. Flow chart of studies evaluating LES for cirrhosis through the selection process

Table 1 summarizes the basic characteristics of the included studies. There were 5 LES compositions according to these eligible studies: (i) CHO (CHO provides >80% energy); (ii) BCAA-CHO (Protein 14%, BCAA 12%, CHO 59%, Lipid 15%); (iii) Protein-CHO (Protein 20-45%, CHO 50%?, Lipid ?); (iv) Coconut milk-CHO (CHO 70%, Protein 2.5%, Lipid 27.5%), and (v) No LES. The included studies were published between 2006 to 2021, most of the studies were from Japan (7 studies) and China (6 studies), while Egypt and Indonesia each had 1 study. A total of 695 participants were included in this review and the average age of the participants was 59.6 years. The mean duration of the intervention was 8.6 weeks.

Six studies compared CHO and no LES with 347 participants. Three studies compared BCAA-CHO and no LES with 89 subjects. Three studies compared Protein-CHO and no LES with 138 patients. Two studies compared BCAA-CHO and CHO with 59 subjects. One study with 35 participants compared Coconut milk and CHO.

The primary outcomes were albumin (Alb), pre-albumin, nitrogen balance (NB), respiratory quotient (RQ), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), ammonia (NH3), cholinesterase (Che), and BCAA/Tyr (BTR). The risk of bias assessments showed that most of these studies were of moderate quality. No publication biases or small sample effects were found.

Table 1. Characteristics of the 15 included study

Comparison	Study	Location	n	Gender (M/F)	Age	Duration	Outcome
(i) CHO (CHO >80%) Vs (v) No LES	Hou W. 2021(22)	China	86	68/18	51.5 ± 11.8	6 months	ALT, AST, TB, Alb, Pre-Alb, Che, NH3, BTR
	Yamanaka-Okumura H. 2010 (23)	Japan	39	28/11	68.1± 9.7	6 months	BMI, AST, ALT, TB, NH3, Alb
	Li P. 2021(25)	China	86	62/24	55.4±4.9	3 months	Alb, Pre-Alb
	Yamanaka-Okumura H. 2006 (29)	Japan	21	47/0	63 ± 10	1 week	AST, ALT, TP, Alb, TB, Che, RQ
	Dong J. 2016 (30)	China	105	-	50.8±8.5	3 months	RQ, TP, Alb, Pre-Alb, Che
	Yu HW. 2012 (31)	China	10	-	42.6±9.7	2 weeks	RQ
(ii) BCAA-CHO (P: 14% P, BCAA 12%; CHO 59%; L 15%) Vs (v) No LES	Takeshita S. 2009 (26)	Japan	56	40/16	69.1±8.2	2 weeks	Alb, AST, ALT, TB, BRT, NH3, Che
	Y. Harima. 2010 (27)	Japan	23	19/4	64.5±9.5	5 weeks	RQ, BRT, Alb, Pre-Alb, ALT, TB, Che, NH3
	Maki H. 2019 (28)	Japan	10	5/5	73.1 ± 8.9	1 month	Alb, AST, ALT, Che, NH3, TB
(iii) Protein-CHO (P: 20-45%) Vs (v) No LES	Ferial. 2014 (15)	Egypt	30	19/11	-	15 days	NH3, NB, TB, ALT, AST. Alb
	Xu J. 2015 (17)	China	83	28/11	68.1± 9.7	4 weeks	AST, ALT, TB, NH3, Alb
	Chen T.2014 (16)	China	25	14/11	49.4±12.7	6 weeks	Alb, Pre-Alb
(ii) BCAA-CHO (P: 14% P, BCAA 12%; CHO 59%; L 15%) Vs (i) CHO (CHO>80%)	Nakaya Y. 2007 (14)	Japan	38	20/18	67±8	3 months	Alb, AST, TB, NB, RQ
	Tatsuki I. 2010 (13)	Japan	21	11/10	66.2±8.2	8 weeks	Alb, AST, ALT, TB, BTR, NH3, Che
(iv) Coconut milk - CHO (CHO 70%, P 2.5%, L 27.5%) vs (i) CHO (CHO 100%)	Suwito Indra. 2015 (24)	Indonesia	35	8/27	54.3±10.6	1 month	Pre-Alb, Alb

Results from network meta-analysis

Albumin

Human serum albumin is a critical plasma protein produced by the liver. Advanced cirrhosis is characterized by reduced albumin concentration as well as impaired albumin function (32).

Data from 13 studies with all five LES compositions were included in the Alb analysis (13-17, 22-29). The network map for Alb in is shown in figure 2. The most common group was the no LES group followed by the CHO group. The most common comparison was CHO versus no LES. The inconsistency test exhibited no inconsistencies in the global analysis, indicating that the direct comparison and indirect comparison results were consistent ($\chi 2 = 0.85$; $p = 0.3556$).

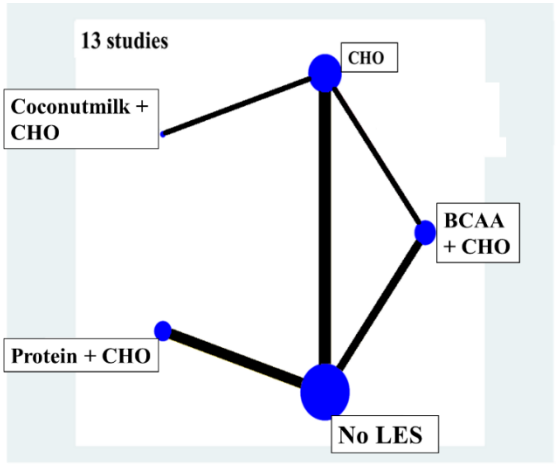

Figure 2. Network map for Albumin.
The nodes (circles) represent the different LES compositions while the edges (lines) represent the available direct comparisons between pairs of LES. Both nodes and edges are weighted by the number of studies involved in each treatment and comparison, respectively.

Figure 3 shows the results of the effects of the LES on Alb. Compared with no LES, Protein-CHO (0.62 g/dL, 95% CI: 0.05 to 1.18) resulted in a significant increment in Alb. There were no significant differences between the other compositions in terms of the effectiveness in increasing Alb.

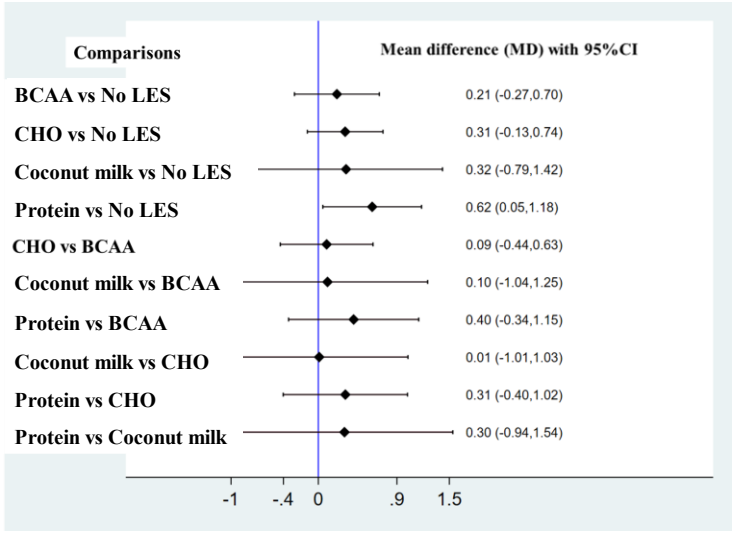

Figure 3. Interval plot for mean difference in Albumin (g/dL) from NMA
**BCAA=BCAA-CHO; Protein=Protein-CHO; Coconut milk=Coconut milk-CHO*

The ranking of treatments for Albumin is shown in table 2. As can be seen, Protein-CHO had the highest probability of being ranked as the best LES composition with 77% of the surface under the cumulative ranking curve (SUCRA). This was followed by CHO and Coconut milk-CHO and then BCAA-CHO.

Table 2. The surface under the cumulative ranking curve (SUCRA) of different LES compositions in various parameters

LES	SUCRA							
	Alb	RQ	AST	ALT	TB	NH_3	BTR	Che
Protein + CHO (P 20-45%, CHO 50%?, L 10%?)	77%		80%	70%	100%	30%		100%
CHO (CHO>80%)	60%	90%	40%	60%	30%	90%	50%	30%
BCAA + CHO (P 14% P, BCAA 12%; CHO 59%; L 15%)	46%	60%	40%	40%	40%	60%	90%	40%
Coconut milk + CHO (CHO 70%, P 2.5%, L 27.5%)	55%							
No LES	10%	0%	30%	30%	30%	20%	0%	30%

Respiratory quotient (RQ)

In patients with liver cirrhosis, glycogen storage in the liver is reduced. Therefore, glucose supply is impaired from nighttime to the early morning fasting state. In this situation, low RQ has been frequently reported.

According to the network map (figure 4), there were 3 LES compositions (CHO, BCAA-CHO, and no LES) from 5 studies (14, 27, 29-31). The overall test for inconsistency was not statistically significant ($\chi2$=3.05, p=0.08).

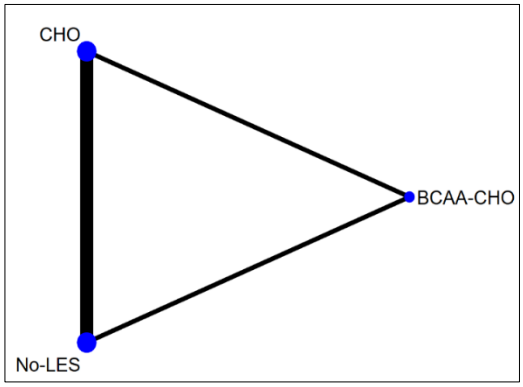

Figure 4. Network map for RQ

From the results of mean difference from NMA, when compared to no LES, CHO and, BCAA-CHO both significantly increased RQ, 0.06, 95%CI: 0.01-0.11; 0.04, 95%CI: 0.02-0.06, respectively.

From SUCRA, CHO (90% area) had a higher probability of improving RQ of liver cirrhosis patients than BCAA-CHO (60% area).

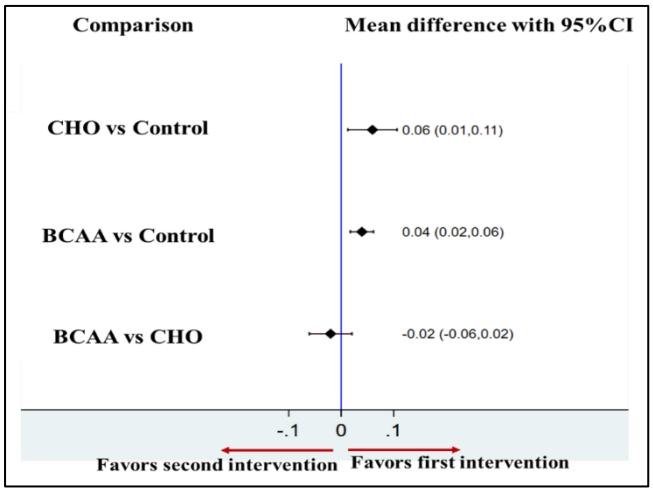

Figure 5. Interval plot for mean difference in RQ from NMA

Alanine Aminotransferase (ALT)

ALT is an enzyme that is concentrated primarily in the liver. In liver cirrhosis, liver cells are damaged which can lead to the leak of this enzyme into the bloodstream and cause an abnormal increment of ALT.

The network map (Figure 6) demonstrates that ALT data was formed by 8 studies with four compositions (CHO, BCAA-CHO, Protein-CHO, and no LES) (13, 15, 22-23, 26-29). The overall test for inconsistency was not statistically significant ($\chi2$=0.3, p=0.586).

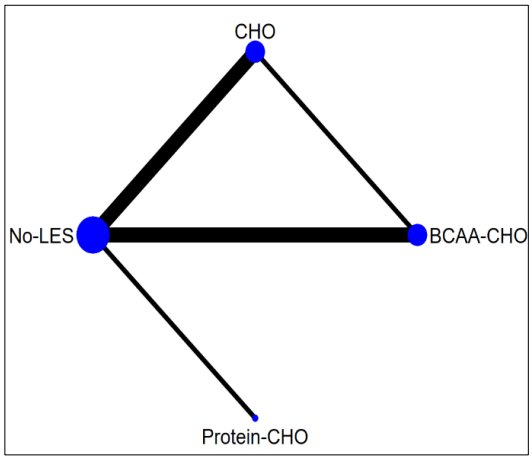

Figure 6. Network map for ALT

The interval plot (Figure 7) indicates that in comparison with no LES, all the compositions tended to decrease ALT; however, there was no statistical significance in any of the comparing pairs.

According to the ranking of SUCRA (table 2), Protein-CHO had the highest probability of being the best in improving ALT, followed by CHO and BCAA-CHO.

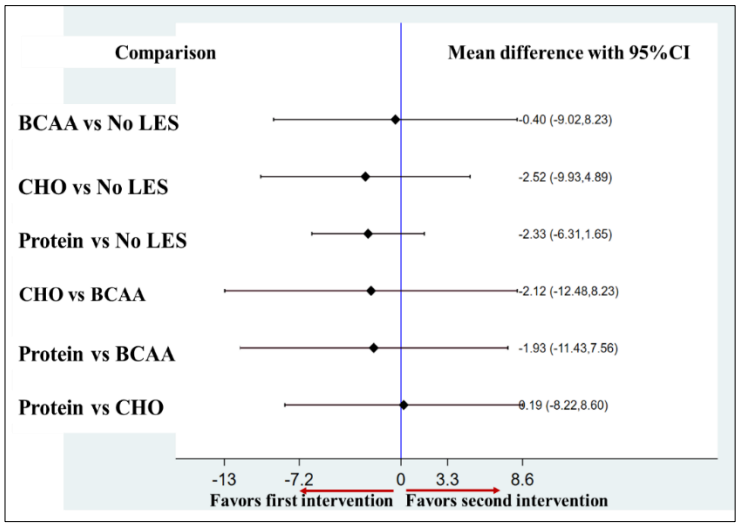

Figure 7. Interval plot for mean difference in ALT (U/L) from NMA

Aspartate transaminase (AST)

Similar to ALT, AST is also an enzyme that is concentrated primarily in the liver and usually elevates in liver diseases.

There were also 8 studies and four compositions (CHO, BCAA-CHO, Protein-CHO, and no LES) that contributed to forming the network map for AST (Figure 8) (13-15, 22-23, 26, 28-29). The inconsistency test showed no inconsistencies in the global analysis ($\chi 2$=0.72, p=0.3951).

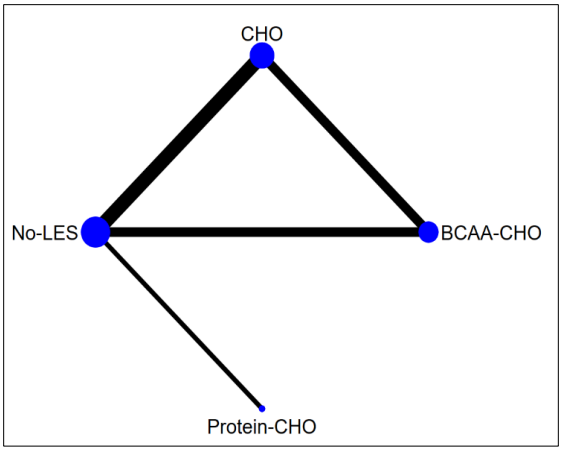

Figure 8 Network map for AST

While there was a decrement in AST, no significant difference was found in the mean difference of AST in all of the comparisons (Figure 9).

Table 2 indicates that Protein-CHO also had the highest probability of being the best in improving AST as well with 80% SUCRA, followed by CHO and BCAA-CHO with both having 40% of the area.

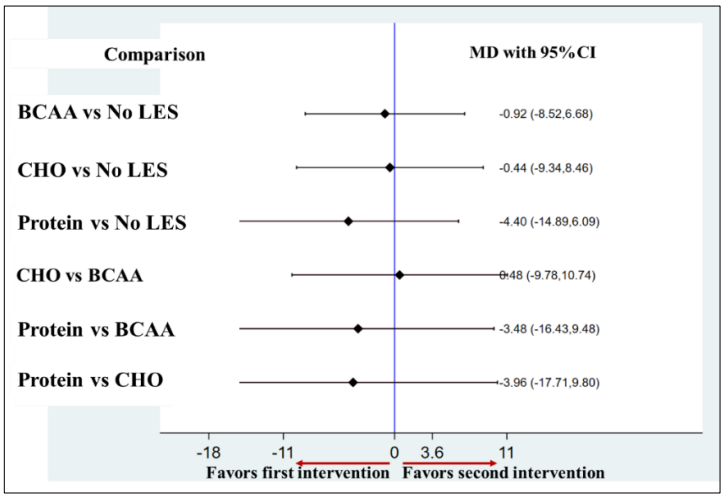

Figure 9. Interval plot for mean difference in AST (U/L) from NMA

Total bilirubin (TB)

The liver normally removes bilirubin from the blood and disposes of it in the stool. But in liver cirrhosis, bilirubin builds up in the blood and can cause jaundice.

The network map was formed from the data of 10 studies including four compositions (CHO, BCAA-CHO, Protein-CHO, and no LES) (Figure 10) (13-15, 17, 22-23, 26-29). There were no inconsistencies in the inconsistencies analysis ($\chi 2$=0.00, p=0.9742).

Figure 11 demonstrates that when comparing to no LES (-1.39mg/dL, 95% CI: -2.32 to -0.46), to BCAA-CHO (-1.32mg/dL, 95% CI: -2.45 to -0.2), and to CHO (-1.38mg/dL, 95% CI: -2.5 to -0.26), Protein-CHO showed great decrement in TB. There was no significant difference in other comparisons.

Protein-CHO with 100% of SUCRA had the highest probability of being the best in decreasing TB compared to others, followed by BCAA-CHO and CHO.

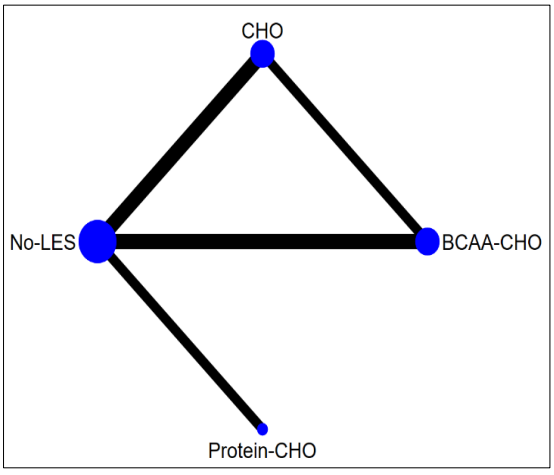

Figure 10. Network map for TB

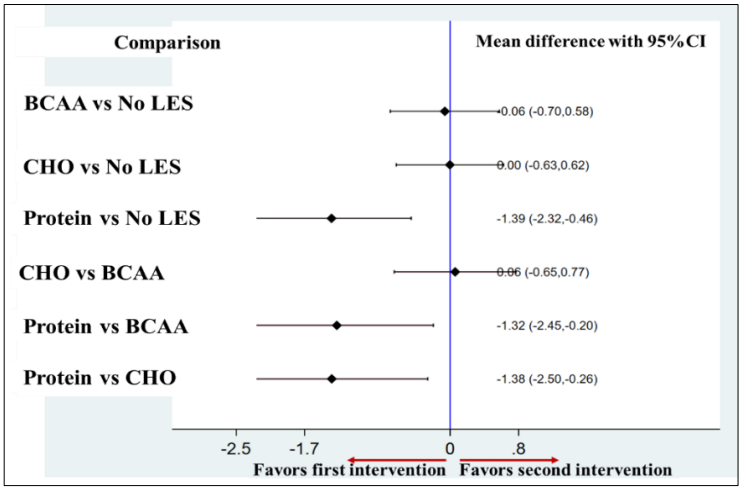

Figure 11. Interval plot for mean difference in TB (mg/dL) from NMA

Ammonia (NH3)

Ammonia (NH3) is a waste product of the digestion of protein. In normal people, ammonia is processed into urea in the liver. In liver cirrhosis, it builds up in the bloodstream which leads to an increased serum NH3.

Data from 4 studies including 4 compositions (CHO, BCAA-CHO, Protein-CHO, and no LES) was presented as the network map for NH3 in figure 12 (13, 26-28). According to the global inconsistencies test, there were no inconsistencies ($\chi 2=0.15$, $p=0.7009$).

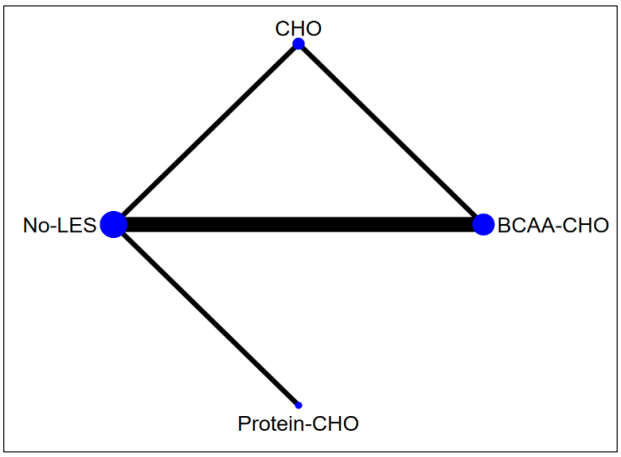

Figure 12. Network map for NH_3

Branched-chain amino acids to Tyrosine ratio (BTR)

It has been reported that BCAA decreases in severe cases of liver cirrhosis patients so BTR (BCAA/Tyrosine) is decreased[6].

Six studies with 3 compositions (CHO, BCAA-CHO, and no LES) provided the data to create a network map for the BTR parameter (Figure 14) (13-14, 22, 28-28). There were no inconsistencies in the inconsistencies global analysis ($\chi 2=2.6$, $p=0.107$).

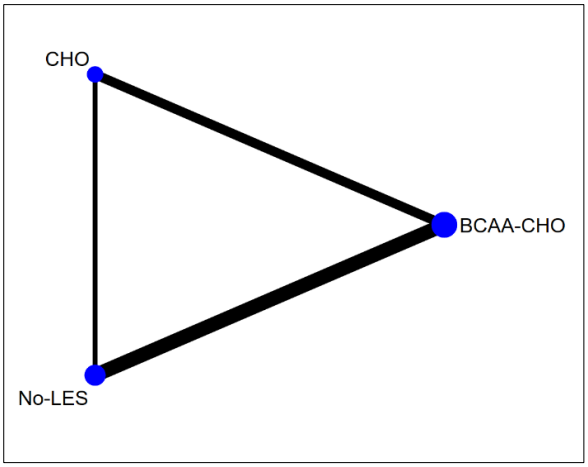

Figure 14. Network map for BTR

The interval plot for the standardized mean difference in NH3 (Figure 13) shows that only the comparison between CHO and no LES had a considerable decrease in NH3 (-0.72, 95% CI: -1.41 to -0.03). CHO also ranks the best in having the most effective area of improving the NH3 status in LC patients (90% SUCRA).

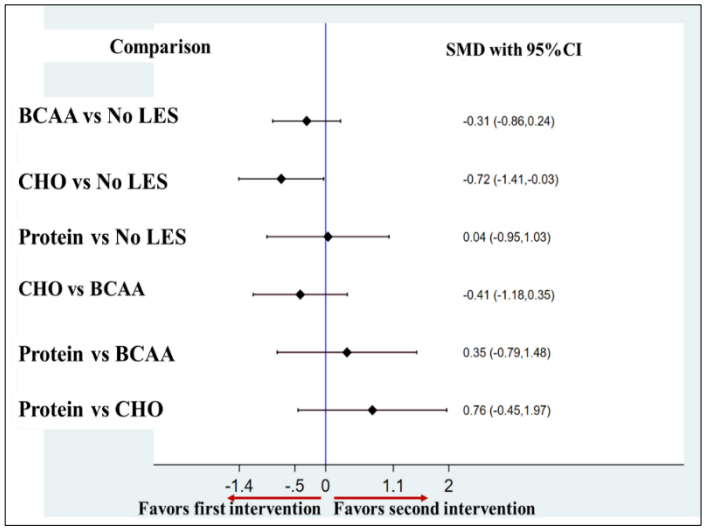

Figure 13. Interval plot for mean difference in NH3 from NMA

From the results of the mean difference of BTR, BCAA-CHO significantly increased BTR compared to no LES (0.63, 95%CI: 0.19 to 1.07) (Figure 15). In addition, the results from table 2 also indicate that BCAA-CHO had a higher probability of increasing BTR than CHO (SUCRA 90%, 50%, respectively).

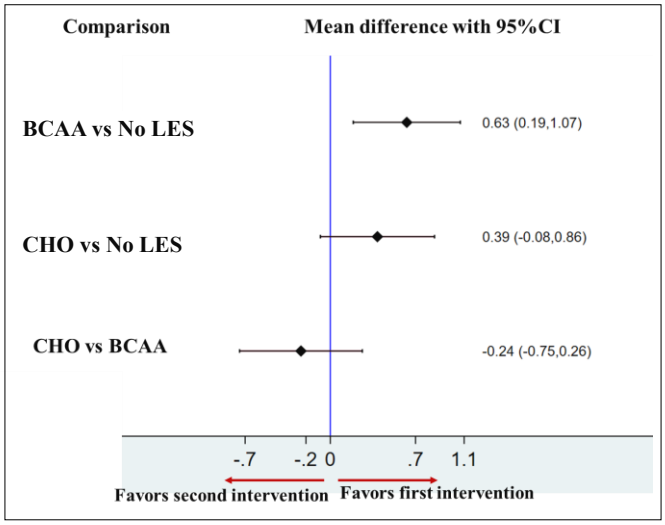

Figure 15. Interval plot for mean difference in BTR

Cholinesterase (Che)

Serum cholinesterase (ChE) is an enzyme synthesized by hepatocytes and its serum levels reflect the synthetic function of the liver. In liver cirrhosis, the Che level is decreased.

9 studies reported on Che involving 4 compositions (CHO, BCAA-CHO, Protein-CHO, and no LES). The data was used to make the network of data for Che (Figure 16) (13, 17, 22-23, 26-30). No inconsistencies were found by inconsistencies global analysis ($\chi 2=0.25$, p=0.6179).

Figure 17 illustrates that when comparing to no LES (3.72, 95% CI: 2.8 to 3.74), to BCAA-CHO (2.98, 95% CI: 2.38 to 3.57), and to CHO (3.22, 95% CI: 2.71 to 3.73), Protein-CHO was more effective in increasing Che. There was no significant difference in other comparisons.

According to table 2, Protein-CHO had the highest probability of increasing Che with 100% SUCRA. BCAA-CHO was ranked second with 40% area and CHO was third with 30% area.

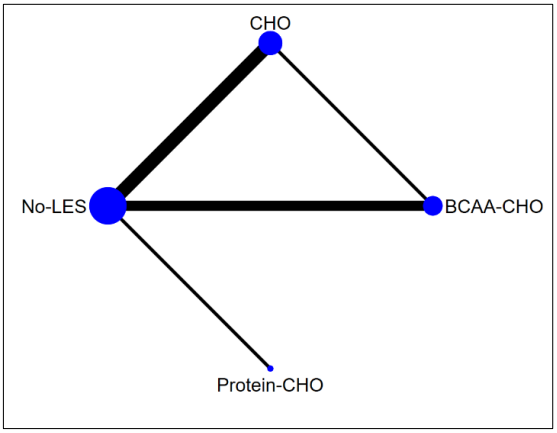

Figure 16. Network map for Che

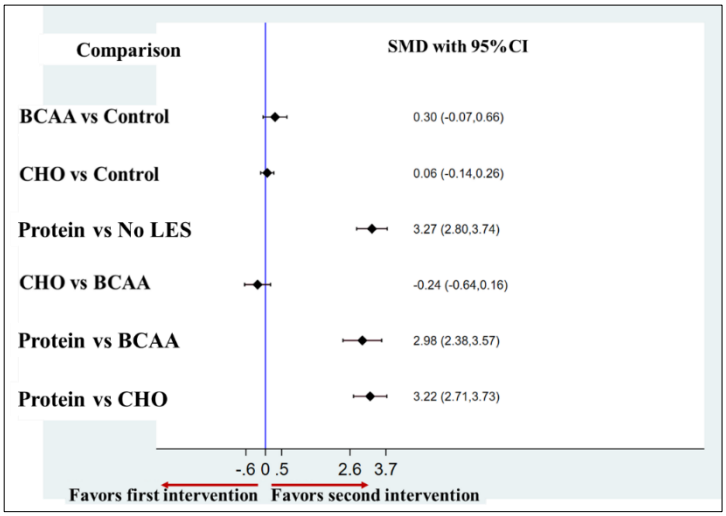

Figure 17. Interval plot for mean difference in Che from NMA

Results from the pairwise meta-analysis

Table 3. Results of the pairwise meta-analysis

	Studies	n	MD (95%CI)	p	I^2
Pre-Albumin					
CHO vs. No LES	4	245	5.45 (-2.5, 13.4)	0.18	96.6%
Protein-CHO vs. No LES	2	108	11.6 (6.37, 16.82)	0.00	96.7%
BCAA-CHO vs. No LES	1	23	-1.26 (-5.35, 2.83)	0.55	-
Coconut milk-CHO vs. No LES	1	17	-0.4 (-12.16, 11.36)	0.95	-
Nitrogen Balance					
CHO vs. No LES	1	19	-0.04 (-2.2, 2.12)	0.97	-
Protein-CHO vs. No LES	1	15	5.47 (1.95, 8.99)	0.00	-
BCAA-CHO vs. No LES	1	19	1.56 (0.02, 3.1)	0.05	-

Pre-albumin (Pre-Alb)

Pre-albumin is the precursor to albumin. It is also made by the liver. So in liver cirrhosis, the level of pre-Alb also decreases.

Pre-Alb was reported in eight out of the 15 studies. Four studies reported comparison between CHO and no LES (22, 24-25, 30); two studies reported comparison between Protein-Cho and no LES (16-17); comparisons between Coconut milk-CHO with no LES and BCAA-CHO with no LES each had 1 study (24, 27).

There was statistical heterogeneity among these trials (I2 = 97%). Using the random-effects model, results indicate that only Protein-CHO had a significant increase in Pre-Alb from baseline (MD = 11.6, 95% CI: 6.37 to 16.82, p = 0.00) (Table 3).

Nitrogen balance (NB)

As mentioned before, with the alternative metabolism of LC patients, especially at night, the protein was used as a source of energy instead of glucose, which increases nitrogen loss. Thus, a negative nitrogen balance was reported in LC patients (32).

Two out of 15 papers investigated the effect of LES on improving NB in LC patients with three different comparisons (CHO vs. no LES, Protein-CHO vs. no LES, and BCAA-CHO vs. no LES) (14-15).

Using the random-effects model, both Protein-CHO and BCAA-CHO showed an increment in NB but only Protein-CHO had a statistical significant (MD = 5.47, 95% CI: 1.95 to 8.99, p = 0.00) (Table 3).

DISCUSSION

Cirrhosis is characterized by a state of accelerated starvation, with an early shift from glucose to lipid utilization for energy during the postabsorptive state. After an overnight fast, lipids account for 75% of the total calories utilized in cirrhotic patients, reflecting increased rates of ketogenesis and gluconeogenesis. In addition, there is an increased consumption of amino acids as a source of energy (protein catabolism) (33). Therefore, many international guidelines have suggested a late evening snack for LC patients (6-7). However, according to a previous study, 77.5% of Vietnamese LC patients did not have the LES and 22.5% of them had LES but with inappropriate nutrient compositions (8). Thus, it is an urgent matter to give Vietnamese LC patients nutrition education regarding LES and know the most appropriate and suitable nutrient compositions concerning acceptability, taste, and finance.

In the previous systematic review conducted by Tsien et al, the formula and dosages of LES were suggested to provide at least 200kcal with 50g of carbohydrates; however, there was no suggestion for the nitrogen source whether it is protein or BCAA (12). LC patients are encouraged to have a high protein diet to prevent a negative nitrogen balance, malnutrition, and sarcopenia. Adequate protein intake has been defined as 1.2 to 1.5 g/kg body weight daily by the ESPEN guidelines (6). In recent years, instead of protein, there has been a surge of interest in the clinical utility of BCAA, mostly in supplemental forms to be included in LES. Some studies have suggested a BCAA-LES over CHO and other compositions (13-14). However, the methodology of these studies is usually a comparison between BCAA, regardless of doses and timing, with control or with CHO and there are no studies directly comparing BCAA with protein as the nitrogen source for LES.

This is the first systematic review and network meta-analysis to examine the effects of different types of LES compositions with the purpose to find the most effective composition. The results revealed that the combination of CHO and protein seems to have the highest probability of improving the nutritional status and liver function of LC patients. Protein-CHO has a higher probability of improving Alb, Pre-Alb, nitrogen balance, ALT, AST, TB, and Che of LC patients than other LES compositions. While late evening snack with predominately CHO has the highest probability of improving RQ and NH3. Lastly, BCAA-CHO contained LES has a higher probability of improving BTR of LC patients than other LES compositions.

In this systematic review, serum albumin, pre-albumin, and nitrogen balance were all significantly increased by Protein-CHO. These biomarkers reflect the synthetic metabolism of liver cells. It is reported that albumin has an important clinical significance in estimating the prognosis of patients with cirrhosis and a better assessment of malnutrition (32). The results of SUCRA of Alb from NMA showed that Protein combined with CHO and CHO alone had been more effective than Coconut milk-CHO and BCAA-CHO. It is worth noting that in the BCAA contained LES, there was 59% of energy from CHO and 14% from Protein as well (Aminoleban EN; Otsuka Pharmaceutical, Tokyo, Japan). It raises the question of whether BCAA is necessary to be provided in LES or not. In the advanced stage of cirrhosis (decompensated liver cirrhosis), a decline in serum BCAA was seen but not in the mild cases (compensated liver cirrhosis) (31). Therefore, the ESPEN guidelines recommend a supplement of BCAA for severe cases of LC (6). Nevertheless, in the studies that used BCAA, all level severity of LC was included and only a few of them offered an iso-nitrogenous regime in comparison (34). Of note, there was a large withdrawal rate (15%) across studies, due to poor palatability, difficulties with compliance with multiple-dose per day, and adverse gastrointestinal symptoms including diarrhea and abdominal distension (34-36). In addition, in many countries like Vietnam, oral BCAA supplements are not available because of the high cost (about 4$/snack). It may be feasible to derive benefits of BCAA from dietary sources rich in BCAA such as chicken breast, beef, salmon, and red bean… (37).

Regarding respiratory quotient (RQ) and BCAA/Tyr ratio (BTR), both of the parameters only included CHO and BCAA-CHO in the comparison as no Protein-CHO studies reported them. The results of RQ indicated that LES helps increase RQ and CHO is maybe more effective than BCAA-CHO. Masahiro et al. reported that survival rate was significantly lower in patients with low non-protein RQ (<0.85) than in patients with scores above 0.85 where 0.85 is the point at which substrate for thermogenesis turns from carbohydrate dominant to lipid dominant (38). This confirms the importance of carbohydrates as the main source of energy for LES and it should be at least 50g as the previous recommendations (6, 12). As for the BTR, BCAA-CHO showed advanced effectiveness in increasing serum BCAA than CHO. However, as there is a lack of evidence from other sources of protein, we could not confirm whether the protein can be as effective as BCAA supplements or not.

The levels of ALT and AST were not significantly different from the baseline when supplying LES in cirrhotic patients. This finding was similar to the systematic review of Guo YJ et al (2018), which suggested that bedtime snacks may not contribute to liver parenchyma damage in patients with cirrhosis in a short time (39). However, in this review, total bilirubin and cholinesterase were significantly improved after the Protein-CHO snack administration. Moreover, ammonia was also successfully decreased by CHO snacks. Thus, with the appropriate compositions of LES which includes both CHO and protein, not only protein synthesis and

energy metabolism can be improved but liver damage can also be managed.

Several limitations in this meta-analysis and network meta-analysis should be considered. First, most studies included in the meta-analysis were single-center studies; furthermore, the sample size in some of the studies was small. Second, the subjects in all searched studies were Asian (most were Japanese). Final, there was no direct comparison between some of the compositions, especially between Protein-CHO and BCAA-CHO. Therefore, I would like to conduct a large, multicenter RCT to confirm the effects of Protein-CHO LES on patients with liver cirrhosis in comparison to an iso-nitrogenous and iso-caloric BCAA contained LES.

CONCLUSION

With the results from the systematic review and network meta-analysis, the recommendable LES compositions should provide at least 200kcal with 50g of carbohydrate. About the nitrogen source, protein is maybe more effective than BCAA in terms of improving protein synthesis and energy metabolism as well as liver parenchyma damage. However, since there is no direct comparison between protein and BCAA as LES, a future study is needed to confirm this finding.

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Original

Development of the Revised Food Frequency Questionnaire for Cambodian School-aged Children

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ABSTRACT: *Background and purpose*. This study aimed to describe the process of revising the Food Frequency Questionnaire (FFQ) developed in 2016 for school-aged children in Cambodia (FFQ-CSC). *Methods*. First, we updated the food composition database using the updated version of the ASEAN Food Composition Table; we also added specific values used in the SMILING food composition tables for Cambodia and Thailand and in the food composition tables for Singapore, Thailand, and Japan. The previous portion sizes were modified based on the current reference amounts. Second, we re-examined the photographs in a picture book and adjusted them according to the revised portion sizes. *Result*. The revised FFQ-CSC was created with a new data entry form in Excel, which allows users to input data more accurately. The new picture book comprises 64 pages for 53 food items and provides respondents with more comprehensive information about various portion sizes. We also created a manual explaining how to use the FFQ-CSC. *Conclusion*. The revised FFQ-CSC with the updated food pictures can be used to assess the dietary intake of Cambodian children. With the newly developed manual, we expect this questionnaire to be used widely and more frequently. It is important to update and revise this tool regularly. Furthermore, the revised FFQ-CSC must be validated and its reliability must be assessed.

Key Words: Cambodia, school-aged children, food frequency questionnaire, revised version, development

INTRODUCTION

The Food Frequency Questionnaire (FFQ) is one of the most commonly employed dietary assessment tools in large epidemiological studies (1). It generally comprises a list of commonly consumed foods with some possible options regarding the frequency of their consumption (2) and can assess habitual food intake over a set time period (3). Several versions of the FFQ have been developed over the last few years (4,5); a validated FFQ can provide information on food intake during a certain period (6,7).

In Cambodia, however, before we developed the FFQ for Cambodian school-aged children (FFQ-CSC) in 2016 to assess their intake of macronutrients and calcium, there was no FFQ to measure their dietary intake. The original FFQ-CSC was validated through a comparison with the 24-hour dietary recall method (hereafter 24-h) developed by the Foundation for International Development/Relief (FIDR) in 2017 (8). Nutritionists usually recommend using or modifying an existing questionnaire in epidemiological surveys (9). Communities should use FFQs that were developed with considerations for how ethnicity, culture, and economic status may affect food intake and dietary habits (10,11). Assigning valid nutritional values to the food items on the quantitative FFQ requires the use of an appropriate dietary database (12) and replacement of the food composition table (FCT) used to calculate nutrient intake, by making appropriate changes to the portion sizes and updating the program code for nutrient calculation (13,14).

Hence, we aimed to modify the FFQ-CSC by using the updated Association of Southeast Asian Nations (hereafter ASEAN) FCT and other food composition data as nutritional data. We also assessed and revised the food items, their frequencies and portion sizes, and the program code to calculate the nutritional values, and provided a picture book that allows children to visualize portion sizes more precisely. In this paper, we describe the process of revising the FFQ-CSC to match the recent dietary trends in Cambodia.

METHODS

Based on the results of our validation study and the current dietary trends, we modified the previous version of the FFQ-CSC (8) as described below.

Data collection

The nutritional data were updated primarily using the 2014 ASEAN FCT (15). The original FFQ-CSC adopted the 2000 ASEAN FCT (16) which was then revised in 2014 (15) and SMILING FCT: Cambodia (17) as its nutrition database. To address the missing data in the ASEAN FCT, we also incorporated other data from Smile FCT: Cambodia (17) and Thailand (18) and FCTs from Singapore (19), Thailand (20), and Japan (21). The nutritional data used in the FFQ-CSC were energy (calories) and nutrients (protein, fat, carbohydrate, and calcium).

Food Items/Frequency/Portion Sizes

Improvements to the FFQ-CSC made in a previous study (8), the current portion sizes in the market were assessed to identify any limitations.

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Program Code for the Nutrient Calculation

We re-created the input sheet on Microsoft Excel 2016 for the revised questionnaire by updating the food items. In addition, a pull-down input was adopted for the frequency and portion size choices.

To assess the quantities of oil and sugar used in the food items, we enabled a system that automatically adds the amount of oil and sugar in proportion to the energy value, instead of requiring respondents to provide these quantities.

Picture Book

We provided a picture book containing the updated representative portion sizes of the food items to help children select their portion sizes easily. The sizes of the dishes and spoons in the photographs were consistent so that respondents could imagine more accurate portion sizes.

RESULTS

Nutritional Data

Table 1 shows the updated nutritional data on the final list of food items.

Food Items/Frequency/Portion Sizes

Table 2 shows the modifications to the nutritional data. Two items (mung bean cake and salt for food) were deleted, one item was modified (salt for young fruits), and portion sizes of all except 17 items (e.g., rice noodles) were revised. The frequency of each item was unified.

Program Code to Calculate the Nutritional Values

We generated a new input worksheet on Microsoft Excel 2016 with pull-down lists for frequencies and portion sizes to enable easy input. The input numbers corresponding to the frequencies and portion sizes were converted into the quantities used to calculate the nutritional values. In addition, automatically calculated amounts of sugar and oil in the food items were included in the program code (Table 2). Based on the following formula, the amounts of oil and sugar per 1000 kcal were calculated to be 21.1 g and 83.9 g, respectively.

Formula: Adding sugar amount (g) per 1000 kcal = (Average carbohydrate intake only from "sugar"/Average energy intake)/S; S is carbohydrate per 100g of sugar (=99.4). Adding oil amount (g) per 1000 kcal = (Average fat intake only from "oil"/Average energy intake)/O; O is fat per 100g of oil (=90.8).

Picture Book

A 64-page picture book was created to supplement the food items listed in the FFQ-CSC. To ensure that the photographs were as realistic as possible, the bowl or plate containing the food was photographed against a background of vertical and horizontal coordinates calibrated in units of length (scale with 2.5 cm × 2.5 cm), and the images were color-printed.

DISCUSSION

We updated the FFQ-CSC based on the results of a previous validation study (8) and the current dietary trends. First, we referred to various FCTs (15, 17-21) to modify the nutritional values of the FFQ-CSC. This method had also been employed for the previously developed FFQs (22-25). In Croatia, the FFQ was developed using a combination of different food databases to analyze the meals because their national food composition database is outdated and impractical to use (25).

Second, regarding the modified food items and portion sizes, in a previous study, 33 food items were added to and 5 food items and beverages were excluded from the FFQ to account for regional and generational dietary changes (14). Another study used altered portion sizes to improve the accuracy of the participants' dietary intakes (13).

Third, the weak correlation between the fat levels in the FFQ-CSC and 24-h (8) was considered. Concerning fat intake, because our survey inquired about respondents' consumption of stir-fried or deep-fried dishes to measure oil intake, it may have been difficult for children to answer these questions (8). A study conducted in Tehran (26) showed that the weak correlation between liquid oil and solid fat might be due to respondents' lack of culinary knowledge. In addition, people use various types of volume measures, such as teaspoons, tablespoons, and cups, for liquid oils and solid fats in food preparations. Therefore, estimating the actual amount of lipids is a challenge. Further, measuring the nutritional values of ingredients in mixed dishes when assessing dietary intake is always a taxing process, and there is a risk of potential loss of dietary information (27). Thus, there was a need to address the question about "oil intake." Similarly, a question about "sugar consumption" in the previous FFQ-CSC used teaspoons as the measuring unit, which may have made it difficult for school-aged children to estimate their sugar intake.

To address these limitations, we developed a new system in which oil and sugar quantities were automatically added per each individual's calorie intake. In addition, we considered that a picture book would help the respondents visualize the food items, making it easier to estimate their portion sizes. An accurate estimation of food portion size remains an important challenge in the collection of dietary data. In China, a food atlas with adequate visual references was developed to improve the accuracy of dietary surveys (28). We should also continue to enhance the food pictures to help respondents accurately determine their portion sizes.

Finally, we emphasize that our next step is to validate the revised FFQ-CSC to ensure its feasibility and reproducibility.

Over the past decade, dietary assessment methods have increasingly made use of online technologies, with the traditional paper-based questionnaires getting replaced (29). An online self-administered FFQ has been validated (30). Digital solutions may minimize some of the errors associated with paper-based questionnaires, and the problem of missing values can be eliminated through automated pop-up reminders and mandatory questions. These in turn may ease respondents' cognitive burden when choosing the right portion size and potentially reduce errors from inaccurate estimations of portion sizes (30). Therefore, we aim to develop an online version of the FFQ-CSC after evaluating the modified FFQ-CSC.

In conclusion, the revised FFQ-CSC with updated food pictures can now be used to assess the dietary intake of Cambodian children. We expect this tool to be used widely and more frequently in Cambodia. However, the validity and reliability of the revised FFQ-CSC must be assessed. Furthermore, we believe that FFQ-CSC should be updated and revised in the future with changing environmental conditions.

Table 1. Question numbers and composition of nutritional data in the previous and updated versions of the questionnaire

Food group	Name of food item	Previous version			Updated version						
		Q. No.	ASEAN FCT 2000	SMILING FCT Cambodia 2013	Q. No.	ASEAN FCT 2014	SMILING FCT Cambodia 2013	SMILING FCT Thailand 2013	Singapore FCT 2003	Thailand FCT 2015	Japan FCT 2020
I. Cereal and cereal products	Boiled rice, rice noodle, sandwich bread, wheat noodle with flavor, corn, sweet potato	Q1–6	6	0	Q1–6	2	4	0	0	0	0
II. Vegetables	Morning glory, amaranth, spinach, cucumber, bean sprout	Q7–11	5	0	Q7–11	1	4	0	0	0	0
III. Fruits	Yellow banana, green banana, coconut cream, guava, green mango, ripe papaya, Spanish plum, rambutan	Q12–19	8	0	Q12–19	7	1	0	0	0	0
VI. Meat, fish, egg, and beans	Fermented soybean, soybean milk, boiled mung bean, roasted peanut, snake head fish, clam, snail, shrimp, crab, canned fish, dried shrimp, duck egg, beef, pork, beef meat ball, chicken, pork blood	Q20–36	16	1	Q20–36	7	9	0	0	1	0
V. Milk and milk products	Fresh milk, yogurt, yogurt drink, cheese, milk powder, ice cream, condensed milk	Q37–43	7	0	Q37–43	6	0	0	0	0	1
VI. Snacks and drinks	Rice cake, snack made from potato or corn, (mung bean cake), Milo powder, Ovaltine powder, soft drink juice, soft drink tea	Q44–50	7	0	Q44–49	4	0	1	1	0	0
VII. Condiments	(Soybean oil), (sugar for food and drink), fish paste in the dish, fish sauce, (salt for food), salt for young fruits*, fermented fish	Q51–58	8	0	Q50–53	0	2	1	0	1	0
All		Q1–Q58	57	1	Q1–Q53	27	20	2	1	2	1

Note: FCT: Food Composition Table; (Foods enclosed in brackets have been removed in the updated version); * Modified in the updated version

Table 2. Frequency of food consumption in the past three months

Item	Changes made in the updated version
Mung bean cake	Removed in the updated version because every participant selected "0" for the frequency question.
Salt	Removed in the updated version due to the difficulty faced by children in answering the question. Moreover, sodium is not a targeted nutrient in the updated FFQ-CSC.
Salt for young fruits	Modified in the updated version to reflect its real use in Cambodian cuisine. It refers to the mixed dipping salt used for eating young fruits, such as mangoes. The nutritional data for "Salt for young fruit" is not available in the FCT. Therefore, it has been modified using nutritional information provided by food companies.
Rice noodle	Portion sizes of rice noodles were adjusted according to a commonly recognized unit of measurement used in the local market or store.
Frequency	In the previous version, for boiled rice, the choice of frequency was set as once a day, 2–3 times, 4–5 times, or 6 times or more per day. However, when revising the questionnaire, we considered potential respondents who never eat boiled rice. Therefore, we set the frequency choice as that for the other items, with ten response categories: never, once a month, 2–3 times a month, 1–2 times a week, 3–4 times a week, 5–6 times a week, once a day, 2–3 times a day, 4–5 times a day, or 6 times or more per day.
Oil	To improve the FFQ-CSC to assess omission of oil, we implemented a system that automatically adds the amount of oil in proportion to energy value, instead of asking about the intake of "oil." The amount is calculated based on the values from a 24-hour dietary recall questionnaire, which was administered in the validation study of the FFQ-CSC [8] by using the following formula: Adding oil amount (g) per 1000 kcal = (Average fat intake only from "oil"/Average energy intake)/O; O is fat per 100g of oil (=90.8).
Sugar	To improve the FFQ-CSC to assess omission of sugar, we implemented a system that automatically adds the amount of sugar in proportion to energy value, instead of asking about the intake of "sugar for food" and "sugar for drink." The amount was calculated based on the values from a 24-hour dietary recall questionnaire, which was administered in the validation study of the FFQ-CSC [8] by using the following formula: Adding sugar amount (g) per 1000 kcal = (Average carbohydrate intake only from "sugar"/Average energy intake)/S; S is carbohydrate per 100g of sugar (=99.4).

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Original

A Crossover Study on the Effects of Continuous Soymilk Intake on Lipid Metabolism and Glucose Metabolism in Vietnamese - Lipid Metabolism Improving Effect –

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ABSTRACT *Background and purpose.* Unprepared soymilk is expected to have a physiological effect due to its high protein content, but there are few clinical trials on soymilk intake in Vietnam and other Asian countries targeting patients with dyslipidemia. This study was conducted a crossover study to verify the effect of unprepared soymilk intake on improving lipid metabolism in a joint study with Vietnam National Institute of Nutrition, Nam Dinh University of Nursing, MARUSAN-AI Co., Ltd., and Kyushu Women's University. *Methods.* It was conducted with the approval of the Kyushu Women's University Ethics Review Committee and the Vietnam National Institute of Nutrition Ethics Review Committee. Thirty-seven type 2 diabetic patients with LDL cholesterol borderline or mild dyslipidemia attending hospital in Nam Dinh City, Vietnam, were included in the analysis. The study design was a randomized crossover study of 2 groups, and only the intervention group was given 500 mL/day of unadjusted soymilk (MARUSAN-AI Co., Ltd.) for 8 weeks, and the washout period was 4 weeks. The primary endpoints were LDL-C, TG and the secondary endpoints were TC, HDL-C. *Result.* The amount of change in LDL-C and TG in the intervention group were significantly reduced at both the 4W-intake and 8W-intake. In addition, the amount of change in HDL-C in the intervention group were significantly increased at the 8W-intake. *Conclusion.* For Vietnamese with type 2 diabetes with dyslipidemia, it became clear that the continuously intake of unprepared soymilk could decrease LDL-C and TG and increase HDL-C.

Keywords: Vietnamese, crossover study, unprepared soymilk, LDL cholesterol, lipid metabolism

INTRODUCTION

In the Socialist Republic of Vietnam (hereinafter referred to as Vietnam), lifestyle-related diseases such as diabetes mellitus, obesity and hypertension are increasing as in developed countries (*1-3*). The proportion of people who died of hyper-LDLemia increased by 37.3% in the 10 years from 2009 to 2019 (*4*), which is thought to be due to increased intake of animal foods and fats and lack of exercise (*5-7*). In many meta-analyses, soy protein intake has been reported to reduce serum low-density lipoprotein cholesterol (LDL-C) and serum triglyceride (TG) (*8,9-14*), and soy globulin is expected to have a physiological effect. Furthermore, β-conglycinin, the main component of soy globulin, has been shown to reduce the accumulation of serum triglyceride and visceral fat (*15*). In recent years, Zhang et al. (*16*) have reported a meta-analysis that fasting blood glucose level, fasting insulin level are significantly reduced by soy protein intake. However, according to a report by Le et al. (*17*) in 2014, the energy intake of Vietnamese people is about 2,000 kcal/day, and the protein intake is 61.6 g/day, but the protein intake from soybean products is 2.8 g/day (less than 5%). Soymilk is one of the soybean products that can easily ingest soybean protein. In particular, unprepared soymilk has a high protein content, so soy protein intake is expected to have a physiological effect. Recently, Ta NT et al. have reported that soy protein intake significantly reduced fructosamine (FRA), total cholesterol (TC), and TG in Vietnamese patients type 2 diabetes mellitus (DM) with hyper-LDLemia in a pre- and post-comparison within the soy protein intake group (*18*) . In this way, it has been suggested that the intake of soy protein may improve lifestyle-related diseases in Vietnamese people.

In addition, the soymilk market share in Vietnam is dominated by prepared soymilk with added sucrose, and the protein content is as low as about 2 g/100mL. Therefore, it is considered that the physiological effect of soy protein cannot be sufficiently obtained. Also, there are few clinical trials in Vietnam and other Asian countries targeting patients with hyperlipidemia due to soymilk intake alone.

In this study, a joint research with Vietnam National Institute of Nutrition (NIN), Nam Dinh University of Nursing (NDUN), MARUSAN-AI Co., Ltd., and Kyushu Women's University, we investigated the physiological effects of continuous intaking unprepared soymilk without sucrose (soy protein content 4.5 g/100mL, carbohydrate content 1.5 g/100mL, MARUSAN-AI Co., Ltd. products) in Vietnamese patients with type 2 DM with

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dyslipidemia. In order to examine whether the lipid metabolism is improved by daily intaking 22.5 g of soy protein contained in 500 mL (250 mL x 2 times) of unprepared soymilk for 8 weeks, a crossover study was performed in two groups, the unprepared soymilk intake group (intervention group) and the prepared soymilk non-intake group (control group). A dietary survey confirmed the effects of diet and consumption of the unprepared soymilk on energy and nutrient intake. For evaluating the effect of continuous intake of raw soymilk on improving lipid metabolism and glucose metabolism, changes in LDL-C, TG, and FRA from before to 4 weeks and 8 weeks after intake were used as primary endpoints. Secondary endpoints were changes in TC, high-density lipoprotein cholesterol (HDL-C), and fasting blood glucose (FPG) levels from before to 4 weeks and 8 weeks after intake.

MATERIALS AND METHODS

1. Ethical considerations

This study was conducted in accordance with the Declaration of Helsinki-Ethical Principles of Medical Research Including the Human Body (World Medical Association) and "Ethical Guidelines for Medical and Health Research Including the Human Body" (Ministry of Health, Labor and Welfare, Japan). The ethics review was approved by the Kyushu Women's University Ethics Review Committee (approval number H30-14) and the Vietnam National Institute of Nutrition Ethics Review Committee. After that, informed consent was given to the subjects, and consent was obtained. The research period is from August 2020 to March 2022.

2. Subjects

The sample size of the subjects was set to forty-four. For type 2 DM patients with borderline or mild LDL cholesterolemia who attending hospital in Nam Dinh City, Vietnam, a screening test was conducted to determine eligibility in 45 patients who gave their consent, and those who met all the following inclusion criteria 1) to 4) and did not meet any of the exclusion criteria 1) to 7) were selected. Inclusion criteria: 1) Patients who are type 2 DM diagnosed in past 5 years with FBG > 7 mmol/L, are using oral drug for management DM, had no severe complications and no advisory of dietetics for controlling DM, have hyperlipidemic with high LDL-C (boundary range: 3.10-3.59 mmol/L, mild range: 3.60-4.10 mmol/L) but not yet taking drugs to control hyperlipidemic. 2) Those who can participate in the test during the test period. 3) Person who can drink soymilk continuously for 8 weeks. 4) A person who has given written consent to participate in this study. Exclusion criteria: 1) Person who are DM and have been used insulin for treatment. 2) Person who are allergic to soybeans. 3) Person with serious disease. 4) Person who has a serious medical history or history of gastrectomy. 5) Person taking internal medicine or formula milk for DM. 6) Excessive alcoholic drinkers or excessive smokers. 7) Any other person who are deemed inappropriate to participate in this study.

The sample size was calculated based on the formula [1] proposed by Hassard.

$$N = 2 \times \frac{(Z_\alpha + Z_\beta)^2 . \sigma^2}{(\mu_1 - \mu_2)^2} \quad [1]$$

$Z\alpha = 1.96$, $Z\beta = 0.84$, σ is the standard deviation of LDL-C concentration, μ_1 is the change in LDL-C concentration from baseline to 8 weeks of taking soymilk, μ_2 is change of LDL-C concentration from baseline to 8 weeks of no taking soymilk. We also assume that σ is approximately 0.245 mmol/L (based on results of our previous research in Vietnamese). Therefore, sample size as calculated as 37 subjects. After adding 20% of dropt out, the suggested sample size is 44 (subjects).

3. Study design and schedule

The study design was a crossover study and was randomly assigned to two groups, group A and group B. The study schedule is shown in Figure 1. In the first period, only Group B ingested unprepared soymilk (MARUSAN-AI Co., Ltd., made in Thailand) 500 mL/day (250 mL x 2 times) for 8 weeks, and the washout period was 4 weeks. In the second period, only Group A ingested 500 mL/day (250 mL x 2 times) of unprepared soymilk for 8 weeks.

The nutritional composition of unprepared soymilk is shown in Table 1. The period before intake of unprepared soymilk hereinafter referred to as "before intake", and 4 weeks and 8 weeks after intake of unprepared soymilk hereinafter referred to as "4W-intake" and "8W-intake", respectively. The measurement items were dietary survey, anthropometry, and blood tests, and the test was conducted once for each period for a total of 6 times.

	1st Period			Washout period	2nd Period		
	Before Intake (0W)	4th week	8th week	4 weeks	12th week	16th week	20th week
Group A	Control group				Intervention group		
Group B	Intervention group				Control group		
Soymilk intake status record	Group B →				Group A →		
Measurement items							
1) Dietary survey	●	●	●		●	●	●
2) Anthropometry	●	●	●		●	●	●
3) Blood tests	●	●	●		●	●	●

Figure 1. Study schedule and measurement items

Table 1. Nutritional composition of unprepared soymilk (test beverage)

Nutrients	Unprepared soymilk (per 100mL)
Energy (kcal)	44
Protein (g)	4.5
Lipid (g)	2.2
Carbohydrate (g)	1.5
Sucrose (g)	0.4
Dietary fiber (g)	0.3

4. Anthropometry

Weight and height were measured while participant standing, wearing light clothing and no shoes. Body fat percentage was measured by TANITA scale (model BC- 541N, TANITA Corporation). Waist circumference was measured by the minimum circumference between the umbilical cord and the iliac crest, and hip circumference was measured by the widest circumference of the buttocks.

5. Blood tests

The subjects fasted from 9 pm the day before blood tests, visited the hospital by 9 am on the day of blood tests (water intake was possible), and had blood drawn. The test items are serum total protein (TP), serum albumin (Alb), FPG, TC, LDL-C, HDL-C, TG, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (Cre), and FRA.

6. Dietary survey

The 24-hour dietary recall method was used for the dietary survey, and a total of 6 surveys were conducted at 0th week, 4th week, 8th week, 12th week, 16th week, and 20th week. Regarding the meals on the previous day, we conducted an interview survey on the meals (type and amount of food) from the time of waking up to the time of bedtime. To answer the intake accurately, we prepared photographs of the actual size of standard vessels (bowl, cup, spoon) used in ordinary households, and conducted an interview survey while showing them. In addition, "Calorie Smile" software (Quest-Computer Co., Ltd.,) which also includes photo data of common dishes, was used during the survey to improve the accuracy of the survey. The calculation of nutrient intake was based on the Vietnamese Food Composition Table published in 2017.

7. Statistical analysis

All values are shown as mean ± standard deviation. Using Microsoft® Excel 2016 for Windows, an unpaired *t*-test were performed on the carry-over effects in Group A and Group B prior to the analysis of a crossover study. The significance level was set to 10%. Statistical analysis software IBM SPSS Statistics 20 (IBM Japan Headquarters, Inc.) was used to analyze the attributes and endpoints of the subjects. Analysis of change between two groups of the same subjects used paired *t*-test and change between two groups of different subjects performed unpaired *t*-test. All tests were two-sided test, and the significance level was set to 5%.

RESULTS

1. Subject selection and attributes

Of the 45 subjects who gave their consent, 42 subjects who met the selection criteria were randomly assigned to each of the two groups, Group A and Group B, by 21. After the start of the study, 5 subjects dropped out due to non-participation in the study (3 subjects) or taking medication for the treatment of dyslipidemia (2 subjects), and finally 19 subjects in group A and 18 subjects in group B, a total of 37 subjects, were included in the analysis (Figure 2). The attributes of the subjects are shown in Table 2. There were no significant differences in age, height, weight, body mass index (BMI), and body fat percentage between the two groups, Group A and Group B.

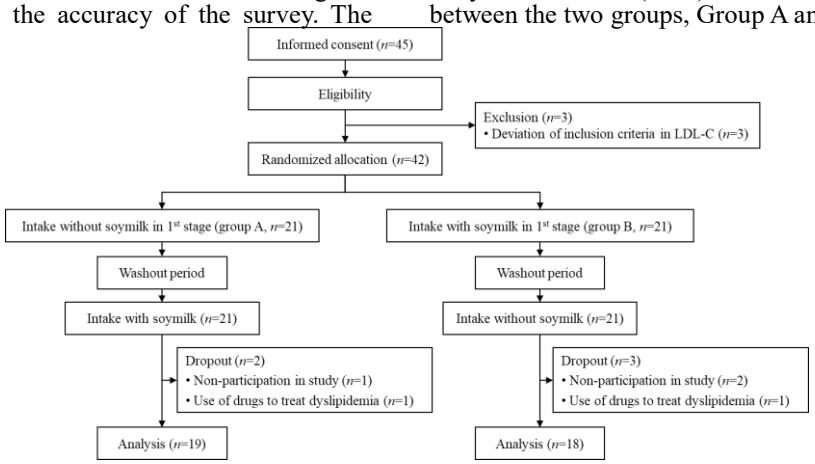

Figure 2. Flow chart from selection of subjects to analysis

Table 2. Subject attributes

	Group A (n=19)	Group B (n=18)	All (n=37)	*p* value†
	Mean ± SD	Mean ± SD	Mean ± SD	
Age (years)	61.8 ± 6.9	64.7 ± 6.3	63.2 ± 6.8	0.21
Height (cm)	155.4 ± 7.7	155.4 ± 6.6	155.4 ± 7.2	1.00
Weight (kg)	58.1 ± 11.6	57.7 ± 7.6	57.9 ± 9.8	0.91
BMI (kg/m^2)	23.9 ± 3.7	23.8 ± 2.5	23.9 ± 3.2	0.93
Body fat percentage (%)	30.9 ± 7.3	30.4 ± 7.2	30.7 ± 7.3	0.83

†: Unpaired t-test, n=37

2. Verification of carry-over effect

As a result of verifying the carry-over effect of the crossover study, LDL-C and TG, which are the primary endpoints of lipid metabolism, had no carry-over effect. However, TC, which is a secondary endpoint, was excluded because it had a carry-over effect. In addition, a carry-over effect was observed in FRA, which is the primary endpoint of glucose metabolism, so it was not possible to analyze glucose metabolism.

3. The amount of change in LDL-C, TG, and HDL-C at the 4W- and 8W-intake

Table 3 shows the amount of change in LDL-C and TG as the primary endpoints and HDL-C as the secondary endpoint at 4W- and 8W-intake. The amount of change from before intake to 4 and 8 weeks of intake period was defined as Δ4W-intake and Δ8W-intake, respectively.

In LDL-C, the control group was 3.43 ± 0.27 mmol/L before intake, 3.66 ± 0.41 mmol/L at 4W-intake, and 3.75 ± 0.53 mmol/L at 8W-intake. The intervention group was 3.46 ± 0.26 mmol/L before intake, 3.09 ± 0.36 mmol/L at 4W-intake, and 2.85 ± 0.32 mmol/L at 8W-intake. The Δ4W-intake in LDL-C was 0.23 ± 0.35 mmol/L in the control group and −0.37 ± 0.30 mmol/L in the intervention group, and the intervention group significantly decreased (p<0.001). The Δ8W-intake was 0.32 ± 0.46 mmol/L in the control group and -0.60 ± 0.28 mmol/L in the intervention group, which was significantly lower in the intervention group (p<0.001).

In TG, the control group was 2.95 ± 1.07 mmol/L before intake, 3.19 ± 1.26 mmol/L at 4W-intake, and 3.01 ± 1.08 mmol/L at 8W-intake. The intervention group was 2.96 ± 1.12 mmol/L before intake, 2.46 ± 0.80 mmol/L at 4W-intake, and 2.38 ± 0.65 mmol/L at 8W-intake. The Δ4W-intake in TG intake was 0.24 ± 0.60 mmol/L in the control group and −0.49 ± 0.64 mmol/L in the intervention group, and the intervention group significantly decreased (p<0.001). The Δ8W-intake was 0.06 ± 0.64 mmol/L in the control group and −0.58 ± 0.79 mmol/L in the intervention group, and the intervention group significantly decreased (p<0.001).

In HDL-C, the control group was 1.14 ± 0.18 mmol/L before intake, 1.14 ± 0.17 mmol/L at 4W-intake, and 1.16 ± 0.21 mmol/L at 8W-intake. The intervention group had 1.17 ± 0.25 mmol/L before intake, 1.21 ± 0.19 mmol/L at 4W-intake, and 1.28 ± 0.19 mmol/L at 8W-intake. There was no significant difference between the two groups in the Δ4W-intake. The Δ8W-intake in HDL-C was 0.02 ± 0.16 mmol/L in the control group and 0.11 ± 0.19 mmol/L in the intervention group, and the intervention group significantly increased (p=0.048).

In addition, the results of the stratified analysis was shown in Table 3. In Δ8W-intake of LDL-C, there was no significant difference between the inner group and outer group in both the control group and the intervention group. In TG, there was no significant difference between the inner group and the outer value group in the control group, but the outer value group showed a significantly lower value in the intervention group (p<0.001). In HDL-C, there was no significant difference between the inner group and outer group in the control group, but the outer value group showed a significantly higher value in the intervention group (p=0.002).

Table. 3. The amount of change in LDL-C, TG, and HDL-C at the 4W- and 8W-intake and stratified analysis of the Δ8W-intake between the inner and outer groups of reference value

Items (unit) (Standard value)	Group		Before intake	4W-intake	8W-intake	Δ4W-intake† Amount of change	Δ4W-intake† *p* value††	Δ8W-intake† Amount of change	Δ8W-intake† *p* value††
LDL-C (mmol/L)	Control	Mean	3.43	3.66	3.75	0.23		0.32	
(< 3.4 mmol/L)	group	SD	0.27	0.41	0.53	0.35	<0.001	0.46	<0.001
	Intervention	Mean	3.46	3.09	2.85	-0.37		-0.60	
	group	SD	0.26	0.36	0.32	0.30		0.28	
Subgroup#								*p* value†††	
Inner group < 3.4 mmol/L (n=17)	Control group	Mean	3.24	3.45	3.52	0.27			
		SD	0.15	0.32	0.43	0.44			
	Intervention group	Mean	3.23	2.91	2.68	-0.56			
		SD	0.11	0.31	0.28	0.29		Control group: 0.57	
Outer group ≥3.4 mmol/L (n=20)	Control group	Mean	3.51	3.75	3.90	0.26		Intervention group: 0.36	
		SD	0.24	0.40	0.53	0.47			
	Intervention group	Mean	3.62	3.26	3.07	-0.62			
		SD	0.20	0.34	0.28	0.26			
TG (mmol/L)	Control	Mean	2.95	3.19	3.01	0.24		0.06	
(1.7-2.25 mmol/L)	group	SD	1.07	1.26	1.08	0.60	<0.001	0.64	<0.001
	Intervention	Mean	2.96	2.46	2.38	-0.49		-0.58	
	group	SD	1.12	0.80	0.65	0.64		0.79	
Subgroup#								*p* value†††	
Inner group < 2.25 mmol/L (n=14)	Control group	Mean	1.98	2.03	2.17	0.20			
		SD	0.34	0.38	0.59	0.64			
	Intervention group	Mean	1.85	1.81	1.86	0.01			
		SD	0.19	0.23	0.16	0.24		Control group: 0.33	
Outer group ≥2.25 mmol/L (n=23)	Control group	Mean	3.55	3.90	3.52	-0.02		Intervention group: <0.001	
		SD	0.91	1.07	0.99	0.63			
	Intervention group	Mean	3.63	2.86	2.69	-0.94			
		SD	0.90	0.76	0.63	0.80			
HDL-C (mmol/L)	Control	Mean	1.14	1.14	1.16	0.01		0.02	
(1.03-1.55 mmol/L)	group	SD	0.18	0.17	0.21	0.14	0.37	0.16	0.048
	Intervention	Mean	1.17	1.21	1.28	0.05		0.11	
	group	SD	0.25	0.19	0.19	0.20		0.19	
Subgroup#								*p* value†††	
Inner group ≥1.03 mmol/L (n=27)	Control group	Mean	1.20	1.20	1.22	0.02			
		SD	0.17	0.16	0.21	0.17			
	Intervention group	Mean	1.28	1.25	1.33	0.05			
		SD	0.13	0.19	0.18	0.14		Control group: 0.89	
Outer group < 1.03mmol/L (n=10)	Control group	Mean	0.98	1.00	1.00	0.02		Intervention group: 0.002	
		SD	0.08	0.10	0.10	0.11			
	Intervention group	Mean	0.87	1.10	1.14	0.27			
		SD	0.26	0.12	0.13	0.23			

†: The amount of change from before intake to 4 and 8 weeks of intake period was defined as Δ4W-intake and Δ8W-intake, respectively. ††: Paired *t*-test, n=37, control group *vs* intervention group. †††: Unpaired t-test, n=37, control group vs intervention group. #: Inner group and outer group mean an inner and outer group the reference value, respectively.

4. The amount of change in Cre, TP, Alb, ALT, and AST at the 4W- and 8W-intake

Table 4 shows the amount of change in Cre, TP, Alb, ALT, and AST, which were conducted as a safety confirmation test. No significant difference was found between the two groups in any of the items.

Table 4. The amount of change in Cre, TP, Alb, ALT, and AST at the 4W- and 8W-intake

Items (unit) (Standard value)	Group		Before intake	4W-intake	8W-intake	Δ4W-intake† Amount of change	Δ4W-intake† *p* value††	Δ8W-intake† Amount of change	Δ8W-intake† *p* value††
Cre (mmol/L)	Control	Mean	83.03	82.94	82.82	-0.13		-0.21	
(62 - 120 mmol/L)	group	SD	16.37	17.14	17.94	7.20	0.94	9.42	0.25
	Intervention	Mean	82.36	82.35	84.29	-0.01		1.94	
	group	SD	16.85	17.89	18.23	4.83		6.33	
TP (mmol/L)	Control	Mean	76.36	76.59	76.54	0.23		0.18	
(3.9 - 6.4 mmol/L)	group	SD	4.24	3.90	4.38	3.00	0.60	3.89	1.0
	Intervention	Mean	76.88	77.52	77.06	0.64		0.18	
	group	SD	4.11	4.64	4.06	3.11		3.43	
Alb (g/L)	Control	Mean	41.69	41.58	41.66	-0.10		-0.03	
(35 - 52 g/L)	group	SD	2.13	1.87	2.10	1.76	0.47	2.03	0.99
	Intervention	Mean	41.70	41.94	41.67	0.24		-0.03	
	group	SD	2.10	2.75	1.87	2.18		1.92	
AST (U/L)	Control	Mean	27.14	28.85	29.42	1.71		2.29	
(< 50 U/L)	group	SD	12.17	19.39	31.21	11.37	0.25	21.85	0.37
	Intervention	Mean	37.81	28.61	33.25	-9.21		-4.56	
	group	SD	78.21	31.17	54.75	47.61		24.47	
ALT (U/L)	Control	Mean	16.29	15.84	16.41	-0.45		0.12	
(< 50 U/L)	group	SD	6.42	5.92	7.37	5.70	0.45	7.72	0.39
	Intervention	Mean	17.87	16.16	16.14	-1.71		-1.73	
	group	SD	13.72	7.02	8.16	8.37		8.07	

†: The amount of change from before intake to 4 and 8 weeks of intake period was defined as Δ4W-intake and Δ8W-intake, respectively. ††: Paired *t*-test, n=37, control group *vs* intervention group.

5. The amount of change in body weight, BMI, waist circumference, and hip circumference at the 4W- and 8W-intake

Table 5 shows the amount of change in body weight, BMI, waist circumference, and hip circumference at the 4W- and 8W-intake. There was a significant difference between the two groups in waist circumference and hip circumference. The Δ8W-intake of waist circumference was 0.83 ± 1.99 cm in the control group and -0.58 ± 2.23 cm in the intervention group, showed significantly lower values in the intervention group (p=0.010). The Δ8W-intake of hip circumference was 0.86 ± 1.49 cm in the control group and -0.05 ± 1.57 cm in the intervention group, showed significantly lower values in the intervention group (p=0.042).

Table 5. The amount of change in body weight, BMI, waist circumference, and hip circumference at the 4W- and 8W-intake

Items (unit)	Group		Before intake	4W-intake	8W-intake	Δ4W-intake† Amount of change	Δ4W-intake† *p* value††	Δ8W-intake† Amount of change	Δ8W-intake† *p* value††
Weight (kg)	Control group	Mean	57.2	57.6	57.4	0.38	0.002	0.17	0.24
		SD	9.6	9.7	9.8	0.68		0.80	
	Intervention group	Mean	57.4	57.2	57.3	-0.18		-0.11	
		SD	9.9	9.7	9.7	0.86		1.15	
BMI (kg / cm^2)	Control group	Mean	23.8	24.0	23.9	0.17	0.002	0.08	0.17
		SD	3.2	3.2	3.3	0.31		0.37	
	Intervention group	Mean	23.9	23.8	23.8	-0.10		-0.07	
		SD	3.3	3.1	3.1	0.46		0.58	
Waist circumference (cm)	Control group	Mean	83.8	84.1	84.6	0.38	0.13	0.83	0.010
		SD	7.8	7.9	8.2	1.80		1.99	
	Intervention group	Mean	83.5	83.2	82.9	-0.30		-0.58	
		SD	7.9	7.8	7.8	1.71		2.23	
Hip circumference (cm)	Control group	Mean	90.4	90.6	91.3	0.21	0.23	0.86	0.042
		SD	5.3	5.3	5.7	1.10		1.49	
	Intervention group	Mean	90.2	90.1	90.2	-0.10		-0.05	
		SD	5.1	5.1	5.1	1.22		1.57	

†: The amount of change from before intake to 4 and 8 weeks of intake period was defined as Δ4W-intake and Δ8W-intake, respectively. ††: Paired *t*-test, n=37, control group *vs* intervention group.

6. The amount of change in nutrient intake at the 4W- and 8W-intake

Table 6 shows the amount of change in energy, protein, lipid, carbohydrate, and sugars at the 4W- and 8W-intake. In Δ4W-intake and Δ8W-intake of energy, lipid, and carbohydrate, there was no significant difference between the intervention group and the control group. In protein intake, Δ4W-intake was -1.4 ± 29.2 g/day in the control group and 21.4 ± 30.1 g/day in the intervention group, and the intervention group significantly increased (p=0.004). The Δ8W-intake was -0.7 ± 29.6 g/day in the control group and 17.8 ± 34.9 g/day in the intervention group, and the intervention group significantly increased (p=0.027). In sugars intake, no significant difference was observed between the two groups in the Δ4W-intake. In Δ8W-intake of sugars was 10.2 ± 18.4 g/day in the control group and 1.4 ± 18.2 g/day in the intervention group, and the control group significantly increased (p=0.040).

Table 6. The amount of change in nutrient intake at the 4W- and 8W-intake

Items (unit)	Group		Before intake	4W-intake	8W-intake	Δ4W-intake† Amount of change	Δ4W-intake† *p* value††	Δ8W-intake† Amount of change	Δ8W-intake† *p* value††
Energy (kcal)	Control group	Mean	1479	1498	1459	19	0.61	-20	0.74
		SD	384	379	375	466		475	
	Intervention group	Mean	1573	1654	1593	80		20	
		SD	428	306	327	475		494	
Protein (g)	Control group	Mean	66.6	65.1	65.9	-1.4	0.004	-0.7	0.027
		SD	20.2	26.5	27.3	29.2		29.6	
	Intervention group	Mean	69.0	90.4	86.8	21.4		17.8	
		SD	24.8	22.7	23.0	30.1		34.9	
Lipid (g)	Control group	Mean	31.5	35.3	30.7	3.9	0.52	-0.8	0.60
		SD	21.7	23.0	18.2	25.8		26.7	
	Intervention group	Mean	37.2	45.5	39.8	8.3		2.6	
		SD	22.2	17.3	18.6	26.9		22.7	
Carbohydrate (g)	Control group	Mean	232.3	230.1	230.1	-2.3	0.40	-2.2	0.39
		SD	68.6	59.0	47.8	78.2		79.0	
	Intervention group	Mean	240.9	220.3	221.6	-20.6		-19.3	
		SD	84.2	60.1	58.4	82.0		84.4	
Sugars (g)	Control group	Mean	11.5	16.7	21.7	5.3	0.21	10.2	0.040
		SD	12.2	19.5	20.7	20.8		18.4	
	Intervention group	Mean	16.7	16.6	18.0	-0.1		1.4	
		SD	16.6	14.6	15.0	18.1		18.2	

†: The amount of change from before intake to 4 and 8 weeks of intake period was defined as Δ4W-intake and Δ8W-intake, respectively. ††: unpaired t-test, n=37, control group vs intervention group.

DISCUSSION

The amount of change in LDL-C and TG in the intervention group significantly decreased at both the 4W- and 8W-intake. In addition, the amount of change in HDL-C in the intervention group significantly decreased at 8W-intake. From the results of these primary and secondary endpoints, the effect of continuous intake of soymilk on improving lipid metabolism was confirmed. Furthermore, as a result of stratified analysis of the Δ8W-intake between the inner and outer groups of reference value in LDL-C, TG, and HDL-C, respectively, in all items, a significant improvement effect was observed in the outlier group of the intervention group.

The American Heart Association (AHA) has reported the mechanism of action of soy protein on serum lipids, such as thyroid hormone and other metabolic hormones, bile acid excretion promoting action, and involvement in LDL receptors (*19*). Also, in 1999, the U.S. Food and Drug Administration (FDA) allowed a soy protein health claim, "Ingesting 25 g of soy protein per day as part of a diet low in saturated fatty acids and cholesterol reduces the risk of heart disease." (*20*) In Japan as well, the cholesterol-reducing effect of soy protein in humans has been recognized, and the Consumer Affairs Agency has approved it as one of the functional substances in Food for Specified Health Uses (FOSHU) (*21*). In addition, it has been reported that triglyceride lowering effect of soy protein is due to the decrease in fatty acid synthesis ability in the liver and the suppression of liver fat accumulation due to the decrease in blood insulin concentration (*22-24*). Although many studies on lipid metabolism of soy protein have been reported so far, few studies have been conducted on the effect of soymilk on the improvement of lipid metabolism disorders, and few studies have been conducted in the Asian region.

In this study, it was confirmed that it can be to reduce LDL-C and TG by continuously unprepared soymilk intake for 8 weeks in Vietnamese patients with type 2 DM who have borderline or mild LDL cholesterolemia. Regarding glucose metabolism, the effect of unprepared soymilk intake could not be clarified because FRA, which is the primary endpoint of glucose metabolism, had a carry-over effect. However, although no significant change in body weight was observed, the amount of change in both waist circumference and hip circumference was significantly reduced at the Δ8W-intake. Waist circumference is adopted as an essential item in the diagnostic criteria for metabolic syndrome in Japan as an indicator of visceral fat accumulation. It is known to increase the risk of which visceral fat accumulation causes hypertension, hyperglycemia, and lipid abnormalities through abnormal adipocytokine secretion, resulting in cardiovascular disease (*25*). In addition, waist circumference has been closely associated with insulin resistance and therefore is recommended as one of the diagnostic criteria for metabolic syndrome by NCEP-ATP III (National Cholesterol Education Program-Adult Treatment Panel III) (*26*). In this study, it found that the continuous intake of unprepared soymilk 500 mL/day for 8 weeks, the waist circumference, which indicates the visceral fat accumulation, was significantly reduced. Therefore, it is suggested that continuous intake of soymilk can be expected to reduce insulin resistance by improving the secretion of adipocytokines, and further research is needed in the future.

Dietary survey revealed that a protein intake was significantly higher in the intervention group than in the control group, both in the Δ4W-intake and in the Δ8W-intake. As 100mL of unprepared soymilk contains 4.5g of protein, it is considered that the intake of 500mL of unprepared soymilk a day significantly

increased protein intake in the intervention group. As mentioned above, it has been reported that soy protein reduces LDL-C and TG and increases HDL-C, and in this study, we were able to obtain similar results from blood tests. Furthermore, despite an increase in energy intake of 80 kcal in the 4th week in the intervention group, the body weight decreased significantly compared to the control group, and waist circumference and hip circumference decreased significantly in the 8th week, respectively. Martinez-Villaluenga C et al. has reported that peptides derived from β-conglycinin, a major component of soy protein, inhibit fatty acid synthase at the molecular level (*27*). It has also been reported that administration of soy protein peptide to rats enhances the thermogenic capacity of mitochondria in brown adipose tissue (*28*). In this way, the results of animal studies have been reported so far, but in this human study, the continuous intake of unprepared soymilk significantly reduced both waist circumference and hip circumference. In the future, we would like to conduct further research to clarify the relationship between the intake of unprepared soymilk and lipid and sugar metabolism.

In conclusion, In this study, it was confirmed that it can be to reduce LDL-C and TG, and to increase HDL-C by continuous intake of unprepared soymilk for 8 weeks in Vietnamese patients with type 2 DM who have borderline or mild LDL cholesterolemia. In addition, it was clarified that the improvement effect was higher in the outer group for TG and HDL-C than in the inner group of reference value. Furthermore, as a new finding, it was revealed that the continuous intake of unprepared soymilk significantly reduced both waist circumference and hip circumference. In the future, we would like to conduct further research to clarify the relationship between the intake of unprepared soymilk and lipid and sugar metabolism.

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CONFLICTS OF INTEREST (COI)

This study will be conducted as a joint research between NIN, NDUN, Kyushu Women's University and MARUSAN-AI Co., Ltd. and will be conducted at a research funding based on a joint research agreement. MARUSAN-AI Co., Ltd. provided research expenses and samples for his COI relationship to be disclosed in this study.

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Original

Phytochemical Content and Antioxidant Activity of Boiled and Fresh Ayo *(Tetrastigma harmandii* Planch.*)* Fruits and Leaves

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Abstract *Background and purpose.* One of the underutilized indigenous plants in the Philippines is Ayo, a vine whose fruits and leaves are used by locals as a souring ingredient in native dishes. The study determined the effect of boiling on the phenolic, flavonoid, tannin, and anthocyanidin content of Ayo fruits and leaves. *Method.* The abilities of the samples to act as antioxidants were evaluated by radical scavenging activity assay. *Results.* Boiling significantly decreased ($p < 0.05$) the phytochemicals and antioxidant activity of the samples, except for the anthocyanidin in the fruits ($p > 0.05$). Generally, fruit samples retained higher levels of phytochemicals during cooking than did leaf samples. The changes in the phytochemical content and antioxidant activity of boiled samples might be due to leaching out of the bioactive compounds from their matrix into the boiling water. *Conclusion.* The study revealed that Ayo could be a good source of phytochemicals, and it exhibited a high radical scavenging activity. Greater antioxidants could be obtained by eating the fruits fresh and raw. When boiling is unavoidable as in the case of the leaves, less water and less cooking time are recommended to retain the optimum benefits of the bioactive compounds present in the plant.

Keywords: Ayo, phenols, tannins, flavonoids, antioxidant activity

INTRODUCTION

The importance of daily and adequate fruit and vegetable consumption in the maintenance of health, and the prevention of non-communicable diseases and malnutrition has been extensively researched, documented, and advocated. Low intake of these crops' accounts for approximately 1.7 million (2.8%) deaths worldwide, and it is estimated to cause around 14% of gastrointestinal cancer deaths, 11% of ischemic heart disease deaths, and 9% of stroke deaths. (1). The report of the joint WHO and FAO expert consultation on diet, nutrition, and the prevention of chronic diseases that took place in 2003 recommends a minimum of 400 g or 5 servings of fruits and vegetables per day. Eating a variety of fruits and vegetables regularly as part of a well-balanced diet provides enough of most micronutrients, dietary fibers, and essential non-nutrient substances needed by the body, thereby encouraging weight management, and reducing morbidity and the risk of mortality (2).
Despite the known health benefits of fruits and vegetable, numerous interventions and dietary guidelines promoting consumption, many populations do not meet the recommended amount. The annual average consumption of vegetables globally is 102 kg per capita, with the highest level in Asia and the lowest in Africa and South America (3). In the Philippines, it is only about 111 g per capita per day or 40 kg per capita per year, far below the requirement set by the WHO (4). Factors that contribute to this trend include cultural issues, impediments in the supply chain, urbanization resulting in inaccessibility to nutritious meals, low socio-economic status, and high costs of produce. Households that lack resources have limited dietary choices since they cannot afford the more popular vegetables in the market (3).
The Food and Agriculture Organization recognizes the urgent need to raise public awareness on the role of underutilized indigenous food resources in countering food and nutrition insecurity. There is also a pressing demand to diversify the food base as a response to climate uncertainty. Underutilized crops are tolerant to drought and natural hazards, and resistant to pests and diseases. They address cultural needs, while being less damaging to the environment. Unbeknownst to many, their nutrient content is much higher than the more popular species that are commonly produced and consumed. Thus, they can be tapped to provide palatable and nutritious foods and increase fruit and vegetable consumption at a minimal cost and effort (5).

Ayo (*Tetrastigma harmandii* Planch.) is an underutilized plant that thrives at low to medium elevations in several provinces of the Philippines. It is a slender vine with branchless, coiled tendrils that is harvested from the wild and used locally as a source of food, medicine, and fiber. Its russet-brown, globose fruits grow in clusters and contain a juicy, sour flesh that may be consumed raw or made into preserves. The leaves are likewise sour and can be utilized as a

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condiment to add flavor to dishes. Both fruits and leaves are typically eaten by the natives with fish (6). Ayo is indigenous in the Philippines, but there is limited data about this plant and there is no literature reporting its nutrient and phytochemical content, and antioxidant capacity. It was therefore imperative to undertake research that would raise awareness and expand the knowledge about this plant. The findings of the study will be useful to future researchers who will be interested in the potential impacts of Ayo on nutrition, and in the product development of Ayo as a jam, jelly, or commercial souring ingredient. Finally, the study would contribute to the documentation of the characteristics of underutilized, indigenous crops and their significant roles in preserving cultures, developing, and sustaining local food systems, and protecting human well-being and health. This study was intended to determine the effect of boiling on the phytochemical content and the antioxidant capacity of Ayo (*Tetrastigma harmandii*) fruits and leaves.

MATERIALS AND METHODS

Sample preparation

Ripe fruits and young shoots of Ayo were collected from Brgy. Culing West, Cabatuan, Isabela. They were transported to the Bio-Assay Laboratory of the Institute of Human Nutrition and Food at the University of the Philippines-Los Baños, where the study would be conducted. The fruits and the leaves were initially cleaned by washing them thoroughly under running water. The rind of the fruits was peeled off. Using a digital weighing scale, two 200 g fruits and two 200 g leaves were prepared before cooking. A portion of the leaves and the fruits were heated to boiling point in a stock pot for three minutes. The fresh and boiled fruits and leaves were placed in a moisture dish, then oven-dried at 45 ˚C overnight. The dried materials were pulverized into coarse powder using a grinder. The dried, powdered samples were transferred into a plastic vial and stored in a desiccator. An extract was obtained by weighing 50 mg of dried, powdered sample and diluting it with absolute methanol to make a 50 ml solution. The solution was swirled for 10 minutes using a vortex mixer, then percolated through a filter paper. The filtrate was decanted into a test tube, and the residue was discarded.

Total phenolic content

The total phenolic content of the samples was determined according to the Folin-Ciocalteu method(7). A 1 ml aliquot was mixed with 1 ml of freshly prepared Folin-Ciocalteu's phenol reagent. After five minutes, 10 ml of 7% sodium carbonate (Na_2CO_3) solution was added, followed by 13 ml of distilled water. The mixture was shaken thoroughly, then kept in the dark for 90 minutes at 23 ˚C. A set of standard solutions of gallic acid (100, 200, 300, 400, 500 µg/ml) was prepared and analyzed in the same manner as the samples. The absorbance was measured at 760 nm against a blank using a UV-spectrophotometer. The results were expressed as mg gallic acid equivalents (GAE) per g of dried sample.

Flavonoid content

The total flavonoid content of the samples was estimated using the aluminium chloride method (7). A 1 ml aliquot was mixed with 4 ml of distilled water, and subsequently added with 0.30 ml of 10% sodium nitrite ($NaNO_2$). After five minutes, the mixture was added with 0.30 ml of 10% aluminium chloride ($AlCl_3$) solution and 2 ml of 1% sodium hydroxide (NaOH) solution, then it was thoroughly mixed. A set of standard solutions of quercetin (25, 50, 75, 100, 125, 150 µg/ml) was prepared and analyzed using the same procedure described initially. The absorbance was measured at 510 nm against a blank using a UV-spectrophotometer. The results were expressed as mg quercetin equivalents per g of dried sample.

Total tannin content

The total tannin content of the samples was assessed by performing the Folin-Ciocalteu assay (7). A 1 ml aliquot was transferred to a 10 ml volumetric flask, then added with 7.5 ml of distilled water, 0.5 ml of Folin-Ciocalteu's phenol reagent, and 1 ml of 35% sodium carbonate solution. It was diluted to 10 ml with distilled water and mixed thoroughly. The mixture was kept at room temperature for 30 minutes. A set of standard solutions of catechin (20, 40, 60, 80, 100 µg/ml) was prepared and analyzed in the same manner as the samples. The absorbance was measured at 700 nm against a blank using a UV-spectrophotometer. The results were expressed as mg catechin equivalents per g of dried sample.

Total anthocyanidin content

The total anthocyanidin content of the samples was determined using the vanillin assay (7). A 1 ml aliquot was mixed with 2.5 ml of 1% vanillin and 2.5 ml of 9 M hydrochloric acid (HCl). It was incubated for 30 minutes at 30 ˚C. A set of standard solutions of catechin (100, 150, 200, 250, 300, 350 µg/ml) was prepared and analyzed following the same procedure described initially. The absorbance was measured at 500 nm against a blank using a UV-spectrophotometer. The results were expressed in mg catechin equivalents per g of dried sample.

Antioxidant activity

The antioxidant activity of the samples was evaluated by performing the 1,-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay (8). A 1 ml aliquot was diluted with 4 ml of distilled water, then mixed with 1 ml of methanolic solution of DPPH. The mixture was incubated in the dark for 20 minutes, after which the absorbance was measured against a blank at 517 nm. The ability of the samples to scavenge the DPPH radical was calculated using the formula:

$$DPPH\ scavenging\ activity\ (\%) = \frac{Absorbance\ of\ control\ -\ Absorbance\ of\ test\ sample\ x\ 100}{Absorbance\ of\ control}$$

Statistical analysis

All functional analyses were carried out in triplicates (n=3). The results were presented as mean ±

standard deviation of three independent observations. Paired sample t-tests were performed to determine whether the differences in the averages were significant. Significance levels were defined at $p < 0.05$.

RESULTS

Total phenolic content

The total phenolic contents of fresh and boiled Ayo were presented in Figure 1. Among the phytochemicals analyzed, phenols had the greatest concentration in all the samples. The phenolic content of the samples ranged from 423.3 mg GAE/g to 551.1 mg GAE/g. The fresh fruits had the highest phenolic content, while the boiled leaves had the lowest phenolic content. After boiling, the phenolic contents of the leaves and the fruits decreased by 23% and 21%, respectively. A two-sample t-test revealed a significant difference ($p < 0.05$) in the phenolic contents of the fresh samples and the boiled samples.

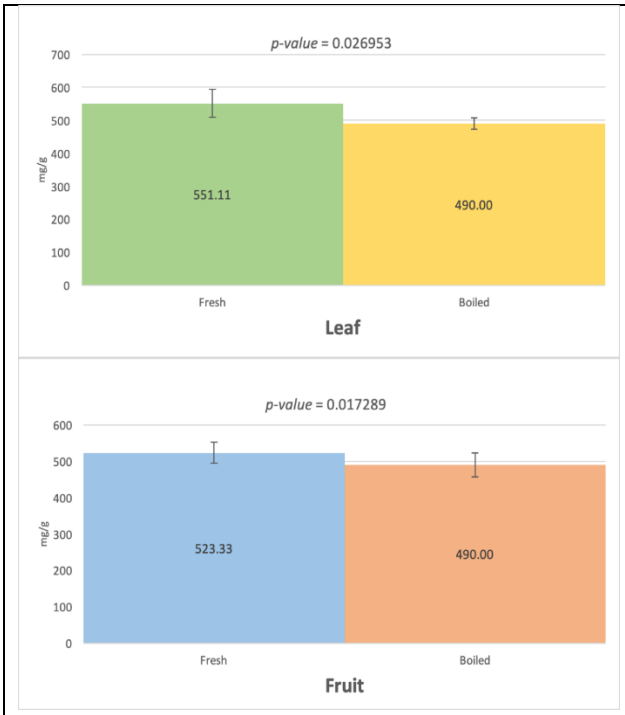

Figure 1. Total phenolic contents of fresh and boiled Ayo

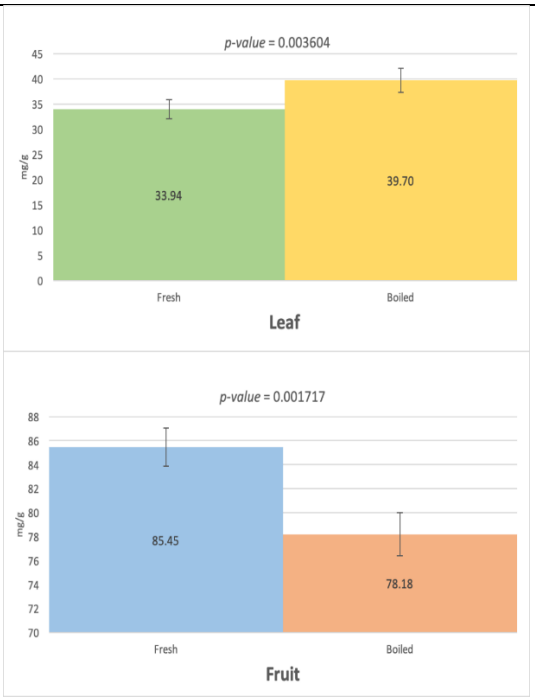

Figure 2. Total tannin contents of fresh and boiled Ayo

Total tannin content

As shown in Figure 2, the tannin concentration of the different samples ranged from 9.09 mg CE/g to 101.82 mg CE/g. Levels of tannins were found to be highest in the fresh fruits and lowest in the boiled leaves. Boiling for three minutes significantly ($p < 0.05$) decreased the tannin contents of the leaves and the fruits by 73% and 23%, respectively.

Flavonoid content

The flavonoid contents of the fruits and leaves before and after cooking were summarized in Figure 3. The flavonoid concentration of the samples varied from 2.17 QE mg/g to 19.90 QE mg/g. The fresh fruits possessed the highest flavonoid levels. On the other hand, the boiled leaves contained the lowest flavonoid levels. The flavonoid concentration of the leaves and the fruits significantly decreased ($p < 0.05$) after boiling.

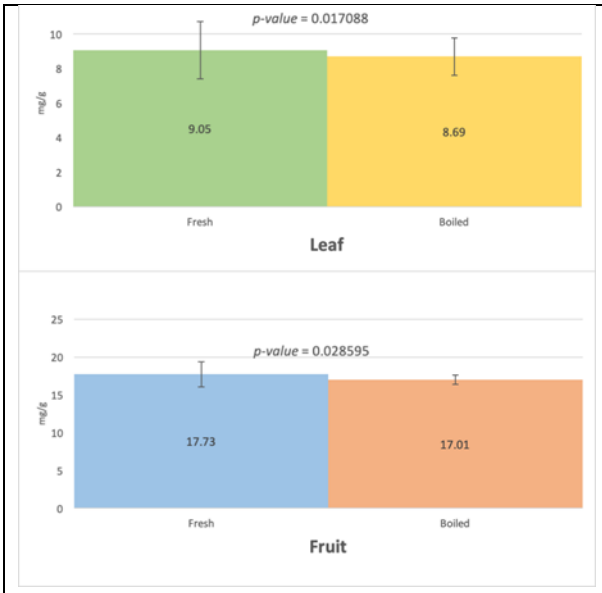

Figure 3. Total flavonoid contents of fresh and boiled Ayo

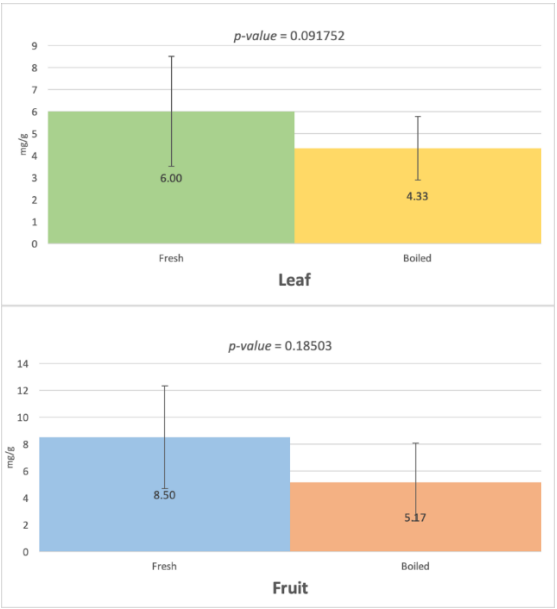

Figure 4. Total anthocyanidin contents of fresh and boiled Ayo

Total anthocyanidin content

Anthocyanidin had the lowest concentration in all the samples. The results presented in Figure 4 suggest that the highest anthocyanidin content was observed in the fresh fruits with 9.33 ± 3.82 mg CE/g dry weight, while the lowest was observed in the boiled leaves with 2.67 ± 1.44 mg CE/g dry weight. After the application of heat, the anthocyanidin content of the leaves and the fruits were reduced by 55% and 44%, respectively. There was a significant difference ($p < 0.05$) in the anthocyanidin content of the leaves before and after cooking, whereas there was no significant difference ($p > 0.05$) in the anthocyanidin content of the fresh and the boiled fruits.

Antioxidant activity

The antioxidant activity of the samples was shown in Figure 5. The greatest radical scavenging activity (94.24 ± 0.08 %) was demonstrated by the fresh fruits, while the least radical scavenging activity (92.89 ± 0.03 %) was exhibited by the boiled leaves. There was a significant difference ($p < 0.05$) in the antioxidant activity of the fresh and boiled samples. The fresh samples had a greater radical scavenging activity compared to the boiled samples. The antioxidant activity may be classified as high (>70%), intermediate (40-70%) or low (<40%) levels of inhibition (9). There was a significant difference in the radical scavenging activity between the fresh leaves and the fresh fruits, and between the boiled leaves and the boiled fruits. Nevertheless, the antioxidant capacities of the four samples were classified as high.

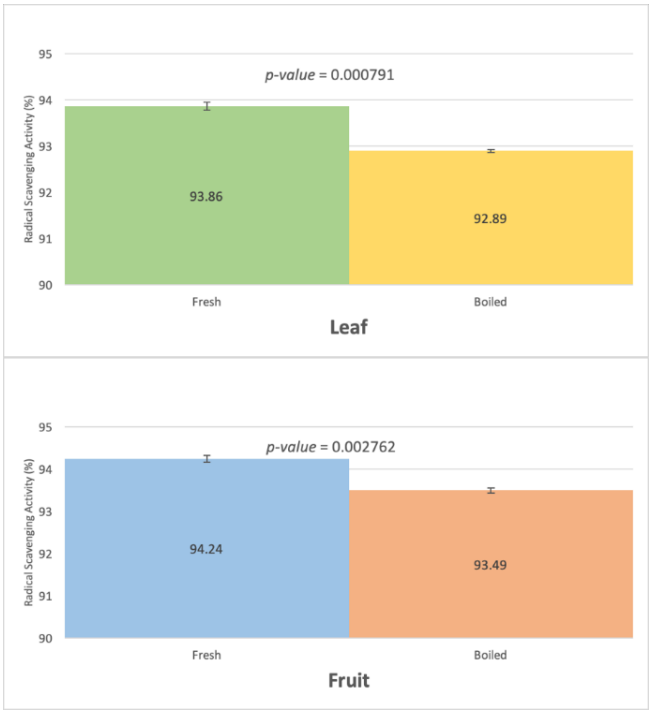

Figure 5. Antioxidant activity of fresh and boiled Ayo

DISCUSSION

Polyphenols are a large group of secondary metabolites present in significant amounts and widely distributed in the plant kingdom, with more than 8,000 structures found in different species. They include simple phenols, flavonoids, tannins, and anthocyanidins, which were the phytochemicals involved in this study. They are synthesized through the phenylpropanoid and polyketide pathways in response to physiological stimuli, stress, ultraviolet radiation, or aggression by pathogens (10). The antioxidant properties of polyphenolic compounds are attributed to the inherent propensity of their hydroxyl groups to dismutate radicals by donating their hydrogen atoms (11). The free radical scavenging activity of the whole molecule is defined by the number of hydroxyl groups it contains (12). Polyphenols are highly reactive and good substrates for different enzymes including glycosidases, esterases, peroxidases, and polyphenoloxidases. They can undergo degradation and polymerization through enzymatic and non-enzymatic reactions during post-harvest storage or food processing, which may result in structural changes and a decrease in their biological activities. The behavior of polyphenols after processing is influenced by several factors such as concentration, type of heat treatment applied, location inside the cell, and interactions with other components of the food (13). The difference in the polyphenolic contents of the fresh and boiled samples in this study could be explained by the fact that polyphenols are polar compounds and leaching occurred during boiling. Polyphenols are also thermo-liable, which means that the greater the amount of water used in cooking, the greater the loss of these compounds (14). Cooking the samples prompted the phenolic compounds to move from uniform distribution in the vacuoles and localize around the cell walls. It also caused the cell-wall components to soften and rupture, which enabled the water-soluble phenolic compounds to be released from the matrix and be dissolved in water (13). According to research on almond skin, the polyphenols decreased during the blanching process, while these compounds increased in the water (15).

Many researches positively correlated the radical scavenging effect with the total amount of phenolic compounds (11); (16); (17). Hence, the decline in antioxidant activity of the boiled samples in this study was a consequence of the leaching process, and structural changes or thermal degradation of phenols, flavonoids, tannins, and anthocyanidins (12). The greater reduction of antioxidant activity in boiled leaves than in boiled fruits could also be due to the larger surface area of the leaves in contact with the water (18). In a study reported, radical scavenging activity of colored peppers considerably decreased ($p < 0.05$) to below 77% of its initial level after boiling for five minutes. It was further reduced to 64% when the boiling time was prolonged to 30 minutes (19). The cooking water was also analyzed, and it was found that there was no significant difference ($p > 0.05$) between the RSA of the raw pepper samples, and the sum of the RSA values in cooked tissues and cooking water after five minutes of boiling. The researchers noted that this was an indication that the antioxidant compounds could have leached into the boiling water.

CONCLUSION

The study determined the effect of boiling on the phenolic, flavonoid, tannin, and anthocyanidin content, and antioxidant activity of Ayo fruits and leaves. The highest concentration for each of the four phytochemicals was consistently observed in the fresh fruits, whereas the lowest amount was invariably noted in the boiled leaves. There was a significant loss ($p < 0.05$) of phytochemical content in the samples after boiling, except for the anthocyanidin in fruits. The antioxidant activity also significantly decreased ($p < 0.05$) in the boiled samples which might be a result of the leaching of the thermo-liable and water-soluble bioactive compounds into the boiling water. In conclusion, maximum antioxidants could be obtained by eating fresh Ayo fruits. However, when boiling is unavoidable as in the case of the leaves, less water and less cooking time are suggested to retain the optimum benefits of the bioactive compounds present in the plant. The water used for boiling could also be consumed in addition to Ayo because soluble bioactive compounds would be present in the boiling water. This study provided some basis for the consumption of Ayo and its possible uses as a functional food or ingredient. Nevertheless, more studies on its proximate composition and vitamin content should be done to obtain comprehensive nutritional properties of this plant.

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