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Stunting and zinc deficiency among 3-5 years old Kankana-ey children in Kibungan, Benguet, Philippines

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ABSTRACT

Introduction: There is a dearth of public health and nutrition information available pertaining to the indigenous groups in the Philippines. Hence, this study described the prevalences of stunting and zinc deficiency, as well as factors associated with zinc status of 3-5 years old children among the Kankana-ey people, an indigenous group living in Kibungan, Benguet, Philippines. **Methods:** A cross-sectional design was used in the conduct of the study where 63 study participants were randomly selected from a chronological list of names of eligible Kankana-ey children by drawing lots. The mothers/primary caregivers of these children were interviewed to collect their socio-economic and demographic data, and dietary intake was assessed through three non-consecutive days 24-hour food recall. Height and weight of the children were also measured to determine their nutritional status. Lastly, blood samples were collected to determine the zinc status of the children based on serum zinc concentration, analysed through atomic absorption spectrophotometer. **Results:** Results showed that 54.0% and 50.8% of the Kankana-ey children had stunting and zinc deficiency, respectively. There was significant positive association between being exclusively breastfed and current zinc status (p=0.001). On the other hand, a statistically significant negative correlation was observed between zinc intake and zinc serum concentration (r=-0.291, p=0.021). **Conclusion:** Undernutrition among Kankana-ey children is a major public health concern in Kibungan, Benguet. Preventive actions must be taken to alleviate the severity of both stunting and zinc deficiency, and to mitigate possible consequences by strengthening the current nutrition and health programmes for indigenous groups, especially among young children.

Keywords: indigenous peoples, nutritional deficiency, nutritional status, zinc

INTRODUCTION

Stunting has long-term effects on individuals and societies, including diminished cognitive and physical development, reduced productive capacity, poor health, and an increased risk of degenerative diseases such as diabetes. On the other hand, substantial evidence shows that zinc plays a critical role in biological processes, including

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cell growth, immunity, and metabolism. Thus, zinc deficiency restricts childhood growth and deprives the immune system, resulting in morbidity and mortality of young children.

Globally, stunting has gained attention due to a multitude of children suffering from its short- and long-term consequences. In 2012, approximately 162 million (25%) under five years old children were affected. These children have an increased risk of dying and suffering from other adverse consequences throughout life (UNICEF, World Bank & WHO, 2013). These numbers are even higher in low- and middle-income countries, particularly in the sub-Saharan Africa, Central Asia, and South Asia regions (Local Burden of Disease Child Growth Failure Collaborators, 2020). In the Philippines, although the national estimates of stunting has declined from 33.4% in 2015 to 28.8% in 2019, it is still considered to be moderately high (DOST-FNRI, 2021). At the provincial level, the estimate for stunting among children under five years old in Benguet, one of the provinces in the Cordillera Administrative Region (CAR) where most of its population are indigenous people, is at 22.7% (DOST-FNRI, 2021). Moreover, zinc deficiency is also a significant problem in the country. The national estimates for zinc deficiency among children under five years old also decreased from 21.6% in 2008 to 17.6% in 2013. However, CAR was included as one of the nine regions where zinc deficiency among children under five years old is considered a high public health concern, with a prevalence of 21.1% (DOST-FNRI, 2015).

Several factors are associated with stunting and zinc deficiency. For stunting, these include parents' education, household wealth index, toilet facilities, birth order (Kamal 2011), feeding practices (Rohner *et al.,* 2013), adequate provision of water and sanitation, febrile respiratory infections, low birth weight, and maternal behaviour (Salvacion, 2017). Similarly, wealth status and dietary adequacy have also been identified as factors associated with zinc deficiency (Goyena *et al.,* 2021; Akhtar, 2013), along with stunting, lack of energy intake, low haemoglobin level, and discontinuing breastfeeding after 12 months (Goyena *et al.,* 2019).

Furthermore, records on the health and nutritional status among indigenous people are limited despite constituting a significant proportion of the country's population. It is estimated that around 10% to more than 20% of the total population are indigenous people, and the majority reside in Mindanao (Asian Development Bank, 2002). The few dated studies on the health and nutrition of this segment of the population showed that preventable and treatable problems persist due to a lack of facilities and resources (Cariño, 2012). Hence, the health and nutrition of indigenous communities have been highlighted in various government programmes, encouraging the participation of non-government agencies and development partners for this effort (Asian Development Bank, 2002). However, other factors such as adverse political conditions and natural disasters continue to affect the well-being of the indigenous people in the country; they are affected by conflict and related injuries, and they are especially vulnerable to the health problems brought about by disasters such as typhoons. Children under five years old in these communities also suffer a higher risk of child mortality (UN-DESA, 2015). In a more recent study, data showed that stunting was significantly higher among under-five-year-old Filipino indigenous group children than their non-indigenous counterparts. There were lower proportions of children who met the recommended energy and other

important nutrients, such as protein, iron, calcium, and B vitamins, in this age group (Duante *et al.,* 2022).

Given the scarcity of data on the health and nutritional status of these communities, public health action planning can be strenuous and could lead to the non-specificity of programmes that meet the needs of the indigenous groups. Hence, this study aimed to determine the prevalences of stunting and zinc deficiency, as well as the factors associated with zinc status of 3-5 years old Kankana-ey children in Kibungan, Benguet, Philippines.

MATERIALS AND METHODS

Study design and participants

A cross-sectional design was used in this study to investigate the magnitude of stunting and zinc deficiency, as well as the factors associated with zinc status of the Kankana-ey young children in Kibungan, Benguet in the CAR. Eligibility criteria included children aged 3-5 years old with no special and/or disease-related conditions, including in-born organ diseases, genetic and hormonal diseases. Mothers and/or primary caregivers of participants served as the respondents of the study. They provided data relevant to the study objectives. The study was conducted in the municipality of Kibungan, Benguet, Philippines from August to September 2016.

Sampling

Of the total number of 353 eligible participants, a sample size of 63 was derived by setting the sample proportion to 0.30, level of confidence to 90%, and margin of error to 10%. Simple random sampling was used to select the 63 participants. The participants were randomly selected from a chronological list of names of eligible children by simple random sampling via drawing lots or lottery method.

Data and sample collection

In this study, there were seven data collection instruments employed and these included questionnaire, weighing scale, height board, centrifuge machine, syringes and vacutainers, Eppendorf tubes, and atomic absorptive spectrometer. A pre-tested, structured questionnaire was developed for this study to collect information on the socio-economic and demographic background of the Kankana-ey children. The indicators used for the socio-economic and demographic profile were age and gender of participants, medical history, and household characteristics (*i.e.,* household size and annual income per capita). Moreover, three non-consecutive 24-hour food recalls, twice for weekdays and once for weekend, were conducted to gather data on the participants' average zinc intake. The mothers or primary caregivers of the children were the respondents for the interviews. For anthropometric measurements, a stadiometer (Seca 206, Hamburg, Germany) and a weighing scale (Model 235 6M, Salter, England, UK) were used to measure the height and weight of the children. Prior to weighing, the scale was calibrated and measurements were taken twice to ensure the precision and accuracy of the data. Data were collected by trained and skilled local enumerators who spoke the language of the participants.

Following the guidelines of the Biochemical Assessment Service Laboratory (BASL) of the Department of Science and Technology – Food and Nutrition Research Institute (DOST-FNRI) for blood sample collection, five millimetres (ml) of blood were collected from the participants through venipuncture. The collection of blood samples was done in the morning by a registered medical technologist. The extracted blood samples were transferred to clean test tubes and were immediately

stored inside an ice chest with wet ice within two hours after collection. The collected samples were centrifuged for 10 minutes at 1500 revolutions per minute (rpm) to separate the serum from red blood cells. The serum samples were then transferred to 1 ml Eppendorf tubes using clean and unused plastic pipettes, and then stored immediately in a freezer at below 0°C. Upon completion of sample collection, the serum samples were transported to BASL for serum zinc analysis by atomic absorption spectrometry. The samples were secured in a water-sealed plastic bag and placed inside an ice chest full of ice packs to make sure that they were kept dry and cool until they reached the laboratory.

Anthropometric and zinc analysis

Data on age and height of the children were used to determine their nutritional status based on cut-off values of the World Health Organization (WHO) Child Growth Standards (WHO, 2006). Moreover, zinc intake of the participants was computed based on the Philippine Food Composition Tables (DOST-FNRI, 1997), and assessed based on the estimated average requirement (EAR) for 3-5 years old Filipino children [*i.e.,* male = 3.3 milligrams (mg), female = 3.2 mg]. Meeting the EAR indicates that a child has met the daily nutrient intake level, which is the median or average requirement of healthy individuals (DOST-FNRI, 2015). As for the analysis of serum zinc concentration, the International Zinc Nutrition Consultative Group or IZiNCG (2004) guidelines were followed in the assessment of zinc status of the children [i.e., <65 micrograms per decilitre (µg/dl) is considered deficient].

Statistical analysis

Descriptive statistics (*i.e.,* frequency, percentage, and mean) were used to summarise the results. Moreover, chi-square test of independence and Pearson correlation coefficient were used in determining the factors associated with zinc status of the Kankana-ey children. A 5.0% value (p=<0.05) was set to test the significance of results. The data in this study were analysed using the Statistical Package for Social Science (SPSS) version 22.0.

Ethical consideration

The study protocols were reviewed and approved by the Cordillera Regional Health Research and Development Consortium – Ethics Review Committee (CRHRDC) and the National Commission on Indigenous People (NCIP). Written and verbal consent were sought from the parents and children before actual data and blood sample collection.

RESULTS

Socio-demographic profile of the Kankana-ey children

Based on the findings, most of the participants fell in the age bracket of 54-60 months, were female, had normal birth weight, were exclusively breastfed, and had completed their recommended vaccination. Most of them belonged to households with five or more members, with an annual per capita income lower than the CAR annual poverty threshold (Table 1).

Prevalence of malnutrition among the Kankana-ey children

The results showed that most participants were stunted, zinc deficient, and had a zinc intake lower than the EAR. These results indicated that there are very high magnitudes of stunting and zinc deficiency among the Kankana-ey children, and these can be considered as a public health concern (Table 2). The mean zinc intake was 3.5 mg, while the mean zinc serum was 66.4 µg/dl (41.0 µg/dl, 98.0 µg/dl).

Table 1. Profile of the Kankana-ey children and their households (*n*=63)

Characteristics	*Frequency (n)*	*Percentage (%)*
Age (months)		
38 – 45	13	20.6
46 – 53	24	38.1
54 – 60	26	41.3
Sex		
Male	29	46.0
Female	34	54.0
Medical history		
Low birth weight (<2500 g)	6	9.5
Exclusively breastfed	33	52.4
Undergone medication/hospitalisation for the past three months	16	25.4
Currently taking medication or food supplement	10	15.9
Completed immunisation	61	96.8
Household characteristics		
Household size		
<5	1	12.7
≥5	55	87.3
†Annual per capita income (<PHP 19,483)	42	66.7

†343.19 USD (1 US dollar = 56.77 PHP)

Association between selected profile of the children and serum zinc levels

The chi-square test of independence revealed that among the socio-demographic characteristics analysed, being exclusively breastfed was the only variable that was significantly associated with zinc status of the Kankana-ey children. Those who were exclusively breastfed were more likely to have adequate zinc serum concentration as they became older children, while

Table 2. Prevalences of stunting, zinc deficiency, and inadequate zinc intake among the Kankana-ey children (*n*=63)

Indicators	*Mean*	*Frequency (n)*	*Percentage (%)*
Nutritional status			
Normal height		29	46.0
Stunted		34	54.0
Zinc status			
Adequate (≥65µg/dl)		31	46.2
Deficient (<65 µg/dl)		32	50.8
Mean serum zinc (µg/dl)	66.4		
Zinc intake			
≥EAR†		26	41.3
<EAR		37	58.7
Mean intake (mg/day)	3.5		

†EAR – Estimated Average Requirement

those who were not exclusively breastfed were more likely to have low zinc serum concentration as they grew older. However, it is noted that there was a higher percentage of 38-45 months old children with adequate or normal zinc status compared to children 54-60 months old. Moreover, there were more children with adequate serum zinc concentration among those who were hospitalised in the past three months. Almost no difference in zinc status was observed among children with complete vaccination. Nutritional status based on height-for-age was also found to have no significant correlation with the zinc status of Kankana-ey children (Table 3).

Furthermore, when the association of zinc intake and zinc serum concentration was analysed, a statistically significant negative correlation (r=-0.291, p=0.021) was found. This result suggests that the Kankana-ey children with high zinc intake tend to have low serum zinc and vice versa.

DISCUSSION

The study findings revealed that although majority of the participants had normal birth weight, a few of them had low birth weight. Birth weight influences child growth and development. It is also a determinant of adult health status (Barker *et al.*, 1993). Low birth weight is defined as an infant having a weight of less than 2500 grams (WHO) and is a risk factor for childhood morbidity and mortality. Based on the 2015 Updating Survey, the low-birth-weight prevalence in Kankana-ey children was lower than the national prevalence (14.0%) (DOST-FNRI, 2015). In terms of infant and young child practices, maternal breastfeeding practices were good. Almost all participants were breastfed, which was close to the national data, confirming 92% of children being

Table 3. Factors associated with zinc status of the Kankana-ey children

Characteristics	*Serum zinc (%)*		χ^2	*p-value*
	Adequate	*Deficient*		
Age (months)				
38 – 45 (n=13)	61.5	38.5	1.292	0.524
46 – 53 (n=24)	50.0	50.0		
54 – 60 (n=26)	42.3	57.7		
Undergone medication/ hospitalisation within the past 3 months				
Yes (n=16)	68.8	31.3	3.278	0.070
No (n=47)	42.6	57.4		
Completed required immunisation				
Yes (n=61)	49.2	50.8	0.001	0.982
No (n=2)	50.0	50.0		
Exclusively breastfed				
Yes (n=33)	69.7	30.3	11.642	0.001*
No (n=30)	26.7	73.3		
Nutritional status				
Normal height (n=29)	41.4	58.6	1.317	0.251
Stunted (n=34)	55.9	44.1		

*Significant at 5% level

breastfed. Breastfeeding is considered an unequalled way of providing ideal food for the healthy growth and development of infants.

The study also showed that one-fourth of the children were hospitalised in the past three months due to fever, diarrhoea, and severe cough. Hospitalisation puts a child nutritionally at risk since it can decrease appetite resulting in poor oral intake, and can induce vomiting and diarrhoea that can cause loss of electrolytes and essential nutrients in the body. Seemingly, the traditional values and culture of indigenous groups influence health and nutrition practices; hence, most children were not hospitalised as some relied on herbal medications, and cultural rituals to alleviate their condition (de Guzman, 2022). As for household characteristics, the Kankana-ey household size was more than the national average of 5. In terms of annual income per capita, almost two-thirds of the household had an income below the 2012 CAR poverty threshold, which is mostly sourced from sales of farm yields.

The study also showed that there was a high magnitude of undernutrition among the Kankana-ey children as more than half of the participants suffered from stunting and zinc deficiency. These findings are comparable with the national and provincial estimates wherein the prevalences of stunting and zinc deficiency among children under five years old are considered a public health concern (DOST-FNRI, 2021; Marcos *et al.*, 2015).

Aside from the high prevalences of stunting and zinc deficiency among young Kankana-ey children, the study also found that zinc status of the children had a positive association with being exclusively breastfed. There is evidence that the introduction of complementary foods before six months of age can lead to a deficiency of vitamins, minerals, and essential fatty acids as this can affect the intestinal mucosal permeability in infants (Silva *et al.*, 2019; Machado *et al.*, 2014). Moreover, the early introduction of food could also increase the risk of diarrhoea, which can further contribute to malnutrition. Still, this does not thoroughly explain the possible relationship of the two variables as there is limited literature available that determines the relationship of past care given during infancy (*i.e.*, exclusive breastfeeding) with serum zinc concentration, which is an indicator of the current state of health and nutrition in children. Perhaps, the relationship between these two variables can be explored and established in future studies.

Contrary to other related studies conducted by Krebs *et al.* (2012), Naupal-Forcadilla *et al.* (2017), and Goyena *et al.* (2020) on the positive association of zinc status and dietary zinc intake among children, this study found a negative correlation between the two variables in Kankana-ey children. The typical meal in the CAR is composed of rice, meat, and vegetables, with meat and meat products as the highest contributors to their protein intake. The region also has the highest consumption of dried beans, nuts, and seeds (DOST-FNRI, 2016), and it was reported that cereals is one of their most consumed food groups. Despite consuming excellent sources of zinc such as meat (Sharma, Sheehy & Kolonel, 2013), the Kankana-ey children may also have possibly consumed a high amount of phytic acid from high consumption of cereals, beans, and nuts, thereby reducing the absorption of zinc among other micronutrients (Gibson, Raboy & King, 2018). Moreover, the current zinc deficiency in soils across the country, particularly in major rice production regions, could have also influenced the zinc status of the Filipino population (Palanog *et al.*, 2019).

Regional initiatives were implemented in partnership with international organisations, such as UNICEF, to combat stunting in Southeast Asia. Scaling Up Nutrition (SUN), a global movement led by 65 countries, aims to end all forms of malnutrition through the implementation of nutrition-specific strategies such as fortification and supplementation, and nutrition-sensitive strategies that focus on access to nutritious foods, clean and safe water, and healthcare services. At present, various national programmes that aim to combat stunting and zinc deficiency are being implemented, which implicitly targets the indigenous population in the country. These programmes include food production/gardening, school feeding, water, sanitation, and hygiene programmes, first 1,000 days programme, micronutrient supplementation, food fortification, and nutrition education for mothers (Herrin *et al.*, 2018; Goyena *et al.*, 2018). However, inaccuracies of nutrition surveillance reports, which are the basis for nutrition monitoring and evaluation of most national nutrition programmes, can result in leakage and under-coverage of nutrition intervention packages, especially among indigenous people (Ramirez, Viajar & Azaña, 2019). Perhaps, the results of this study could be used in an effective targeting system for nutrition intervention programmes.

To our knowledge, this study is the first to provide an overview of the prevalences of stunting and zinc status of the Kankana-ey children in Kibungan, Benguet. Its findings highlight the need for a concerted effort against the prevailing nutrition problems among this population. It should help public health practitioners and policy makers in developing and strengthening the current strategies to improve the nutrition and overall health of the indigenous people, particularly among young children. Ample investment and resources are needed to reach all families and children, especially the indigenous people who are often sidelined in nutrition programmes. Moreover, more in-depth studies should be conducted to provide a complete picture of the nutrition situation of the Kankana-ey people in order to develop a more effective and appropriate public health approach.

CONCLUSION

The prevalences of stunting and zinc deficiency among 3-5 years old Kankana-ey children in Kibungan, Benguet are of public health significance. Underlying factors associated with the zinc status of the children were past care (*i.e.,* being exclusive breastfed) and dietary zinc intake. These results indicate that public health interventions are imperative to improve the nutrition and overall health of the Kankana-ey children. Strategies may include strengthening programmes on the promotion of breastfeeding, multiple micronutrient supplementation, accurate periodic growth monitoring, and nutrition education for mothers to improve their child's nutritional status, especially among indigenous groups.

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Authors' contributions

JSP, principal investigator, conceptualised and designed the study, led the study data collection, prepared the draft of the manuscript, and reviewed the manuscript; NPG, LSA, CVCB, JTD, advised on the study design, data analysis and interpretation, and reviewed the manuscript; JPA, KVM, contributed to the data analysis, drafting of the manuscript, and reviewed the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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Effects of soaking duration and incubation conditions on GABA biosynthesis in MangBuk brown rice of Vietnam

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ABSTRACT

Introduction: Many people are currently interested in improving and maintaining their health status by changing their dietary habits, like eating more natural foods; thus sprout products are becoming increasingly popular. In this context, sprouted brown rice grains are an excellent example of functional food, because besides their nutritive value, they also lower the risk of various diseases and/or exert health-promoting effects. In this paper, we focused on the bioactive compound γ-aminobutyric acid (GABA) in germinated brown rice. GABA is known as an important amino acid that can help reduce hypertension and inhibit cancer cells development. **Methods:** We investigated the hydration characteristics of brown rice by drying them in a moisture analyser at 130°C until reaching a constant weight. The effects of soaking (duration and pH of soaking solution), as well as incubation conditions (temperature and time) on GABA biosynthesis in MangBuk brown rice of Vietnam were measured. Quantification of GABA was measured using a spectrophotometer. **Results:** GABA content in MangBuk type 1 brown rice was higher than in type 2. GABA content reached its highest value at 691.88 µg/g for type 1 rice and 596.48 µg/g for type 2 rice when MangBuk brown rice was soaked in a pH 7 water at 30°C for 12 hours, and then incubated at 35°C for 30 hours in aerobic condition. **Conclusion:** Germination conditions modified the content of biologically active compounds in MangBuk soft and hard rice varieties. GABA was synthesised during germination based on three factors, namely time of incubation, temperature of incubation, and pH of solution.

Keywords: gamma-amino butyric acid, germinated grains, hydrolytic enzymes, MangBuk brown rice, soaking duration

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INTRODUCTION

MangBuk red rice (brown rice) is a traditional rice of the Xe Dang people in the upland commune of MangBuk, Kon Tum province, Vietnam, which has been preserved from generation to generation. This rice has only two varieties: soft rice and hard rice. Every year in April, the Xe Dang people start to plant rice and wait until October to harvest it. Strangely, red rice plants like to live on arid land, without watering, and enjoy only rainwater from the beginning to the end of the season. The planted rice grows and develops wildly by itself.

Brown rice is an intact whole rice grain obtained after peeling off its rice husk. It contains more nutritional components, such as dietary fibre, phytic acid, vitamin E, and vitamin B than ordinary milled rice. These bio-functional components exist mainly in the germ and bran layers, most of which are removed by polishing or milling. In germinated grains, hydrolytic enzymes are activated and they decompose starch, non-starch polysaccharides, and proteins, which leads to an increase in oligosaccharides and amino acids (Patil & Khan, 2011; Albarracin *et al.*, 2019). The decomposition of high molecular weight polymers during germination leads to the generation of bio-functional substances and the improvement of organoleptic qualities due to the softening of texture and increase in flavour. Germinated rice is produced by soaking it in water at an appropriate temperature and for the right duration. The result yields a 0.5-1 mm long sprout from the brown rice grain; at this stage nutrient accumulation in the grain is at its maximum. Manufactured germinated rice is mostly sold in dried form (the drying does not affect the superior nutritional value accumulated from germination), which looks very similar to ordinary brown rice. The goal of the drying process is to prolong the shelf life of germinated rice (Albarracin *et al.,* 2019). Compared to other rice varieties, germinated brown rice offers considerable health benefits, thanks to its increased amounts of γ-aminobutyric acid (GABA), dietary fibre, inositols, ferulic acid, phytic acid, tocotrienols, magnesium, potassium, zinc, γ-oryzanol, and prolylendopeptidase inhibitor. Additionally, the process of germination enhances the bio-availability of nutrients by neutralising phytic acid and releasing proteins, vitamins, and enzymes that allow important nutrients to be absorbed during digestion (Patil & Khan, 2011; Albarracin *et al.,* 2019). The germination of brown rice also helps free its bound minerals, making them more absorbable by the body; besides, the rice also becomes more tender and tastier (Kayahara & Tsukahara, 2000).

GABA is a non-protein amino acid widely distributed in nature that is a neurotransmitter in the brain and spinal cord of mammals (Bown *et al.,* 1999). GABA health benefits include: diuretic effects, tranquillising effects (Jakobs *et al.,* 1993), Alzheimer's disease prevention (Ito & Ishikawa, 2004), regulation of blood pressure and heart rate, pain and anxiety relief (Kono & Himeno, 2000), improved insulin secretion to prevent diabetes (Huang *et al.,* 2007), or inhibition of cancer cell proliferation (Oh & Oh, 2004). GABA is produced primarily by the decarboxylation of L-glutamic acid, catalysed by the glutamate decarboxylase enzyme (GAD) during the germination process of brown rice (Bown *et al.,* 1999). The amount of GABA in germinated brown rice in Japan was noticed to be ten times more as compared to milled white rice and two times more than that of brown rice (Kayahara & Tsukahara, 2000). Roohinejad *et al.* (2009) reported that GABA content after germination in Malaysian brown rice seeds ranged between 0.01 and 0.1 mg/g. Based on that, we studied in this

paper what are the conditions that give the highest GABA content in MangBuk red rice.

MATERIALS AND METHODS

Materials

The two varieties of MangBuk brown rice were taken from Mang Den village, DakLong commune, Kon Plong district, Kon Tum province, Vietnam. The raw rice materials were two types of brown rice of the same origin, but different in characteristics. Type 1 brown rice was soft rice, while type 2 brown rice was hard rice.

Analysis of hydration characteristics of brown rice

Brown rice was washed with fresh water to rinse out contaminants and then thoroughly rinsed with distilled water. Type 1 and type 2 brown rice were divided into five portions (20 g/sample) for each type. Brown rice samples were soaked in distilled water (pH 7) with a ratio of 1:2 (w/v) in an incubator (Wise Cube, DAIHAN-Korea) at 30°C for 6, 8, 10, 12, and 14 hours. At each time interval (duration), the moisture content of the brown rice samples was analysed by drying them in a moisture analyser (AND MX-50, Japan) at 130°C until reaching a constant weight.

Analysis of GABA content in germinated rice at different pH of soaking solution

The brown rice was soaked for the durations mentioned above. Type 1 and type 2 brown rice were divided into three portions (20 g/sample) for each type. Brown rice samples were soaked in water (1:2) (w/v) with adjusted pH levels of 5, 6, and 7 in an incubator at 30°C with the soaking duration chosen based on the results of the previous experiment (12 hours). For each pH, the GABA content of brown rice samples was analysed using the absorption spectrophotometry method (UV-VIS 2502, LaboMed, Inc).

Analysis of GABA content in germinated rice at different incubation durations

The brown rice was soaked in water with a pH chosen based upon the results of the previous experiment. Type 1 and type 2 brown rice were divided into six portions (20 g/sample) for each type. After being soaked in water at 30°C for 12 hours (duration chosen from the previous experiment), brown rice samples were stored in Erlenmeyer flasks. They were then incubated with water (1:0.5) (w/v) at pH 7 without lids (aerobic) and with lids (anaerobic) in an incubator set at 30°C for 18, 24, and 30 hours of duration.

With lids, the Erlenmeyer flasks were airtight and thus the samples lacked oxygen. Without lids, the samples had access to oxygen throughout the incubation duration. At each incubation time interval, GABA content in brown rice samples was analysed by absorption spectrophotometry (Karladee & Suriyong, 2012).

Analysis of GABA content in germinated rice at different incubation temperatures

The brown rice was incubated for a duration chosen based on the results of the previous experiment. Type 1 and type 2 brown rice were divided into 6 portions (20 g/sample) for each type. After being soaked in water with adjusted pH 7 at 30°C for 12 hours, brown rice samples were stored in Erlenmeyer flasks, then incubated with water (1:0.5) (w/v) at pH 7 without lids and with lids in an incubator set at each tested temperature (30, 35, and 40°C) for 30 hours. For each incubation temperature, GABA content of the samples was analysed by absorption spectrophotometry.

Determination of GABA content

Determination of GABA content was based on the assay of Karladee and Suriyong (Karladee & Suriyong, 2012). Whenever measurement was needed to be taken, germinated rice was grounded to fine powder after soaking and incubation. The powder was diluted with distilled water with a ratio of 1:1. Then, 10 ml of the sample and 0.53 g of Na_2CO_3 were added in a 50 ml falcon tube. After that, the mixture solution was centrifuged at a rotational speed of 5000 rpm for 15 minutes. Following that, all residues and other components of the centrifuged mixture were removed by a filter paper. The supernatants were then collected and taken to quantify GABA content as follows: 0.6 ml of supernatants was mixed with 0.4 ml borate buffer (pH 0.9) and 2 ml phenol 6.0% (w/v). Next, the mixture was shaken vigorously for one minute and cooled in water at 20°C for five minutes. 1.6 ml NaOCl 8% was added and boiled at 100°C for ten minutes, then cooled in water for five minutes. Finally, GABA content was measured with an absorbance of 630 nm wavelength on a spectrophotometer.

Statistical analysis

Each treatment was repeated three times. All parameters were analysed by analysis of variance (ANOVA) using IBM SPSS Statistics for Window, version 20.0 (IBM Corp, Armonk, New York, USA).

RESULTS AND DISCUSSION

Hydration characteristics of brown rice

Soaking duration affected the germination ability of brown rice. The study results in Figure 1 show that for both types of brown rice, during the first six hours of soaking, seeds absorbed moisture very quickly, increasing the moisture content from 11% to 30% due to the difference in moisture content inside and outside of brown rice. In addition, during this stage, there was

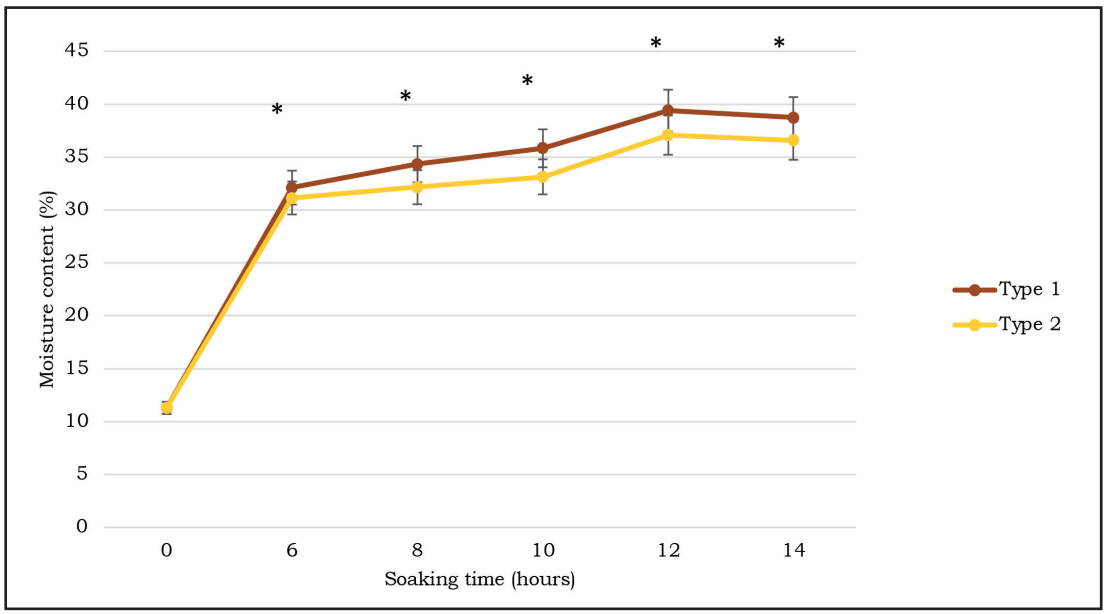

Figure 1. Hydration characteristics of MangBuk soft rice (Type 1) and MangBuk hard rice (Type 2) during soaking at 30°C

*Statistically significant differences between different times of incubation (p<0.05)

Table 1. GABA content in two varieties of germinated brown rice (MangBuk type 1 and 2) in different soaking solutions

Type of brown rice	*pH*	*GABA content (µg/g)*
Type 1	Brown rice (control)	20.76±0.72^{f}
	pH 5	155.03±14.31^{d}
	pH 6	216.85±8.02^{b}
	pH 7	284.60±3.77^{a}
Type 2	Brown rice (control)	16.96±2.56^{f}
	pH 5	40.84±10.31^{e}
	pH 6	183.02±8.92^{c}
	pH 7	227.38±9.60^{b}

Values represent *means±SEM* from 3 different experiments.
^{abcdef} Different letters in the column indicate significant differences between control and treatment at $p<0.05$.

a quick absorption into the embryo of the kernel (Bello, Tolaba & Suarez, 2004). After that, the dehumidification process slowed down and reached a saturation state after 12 hours for both rice types. When the soaking duration was increased from 12 to 14 hours, the absorption of water in the grain changed insignificantly. In this phase, water was absorbed in a constant linear rate until mass transfer reduction, when equilibrium was reached, correlating with grain metabolism and the visible onset of germination. This phase can be correlated to the filling of voids in the grains by water molecules and the lack of chemical links between grain constituents and water (Borges *et al.,* 2017). Many previous studies have shown that a humidity of about 35% - 40% (w/w) would ensure normal seed germination (Cung *et al.,* 2013; Komatsuzaki *et al.,* 2007). Therefore, the soaking process should end after 12 hours for both rice types. The moisture content at this moment was 39.4% for type 1 brown rice and 37.0 % for type 2 brown rice.

Table 2. GABA content of brown rice incubated in aerobic and anaerobic conditions for different durations

Incubation duration (hours)	*GABA content (µg/g)*	
	Aerobic incubation	*Anaerobic incubation*
Type 1 brown rice		
Brown rice (control)	20.76±0.73^{g}	20.76±0.73^{g}
18	420.73±9.55^{d}	397.31±2.62^{e}
24	482.90±9.48^{b}	437.57±9.39^{c}
30	538.19±5.91^{a}	517.04±7.58^{a}
Type 2 brown rice		
Brown rice (control)	16.96±2.56^{g}	16.96±2.56^{g}
18	351.66±8.91^{e}	310.75±9.97^{f}
24	437.00±6.70^{c}	412.53±7.53^{d}
30	478.44±12.12^{b}	462.87±6.15^{b}

Values represent means±*SEM* from 3 different experiments.
^{abcdefg} Different letters indicate significant differences between control and treatment at $p<0.05$.

Effect of pH of soaking solution on GABA content in rice germination

The GABA content of germinated brown rice with various soaking solutions is shown in Table 2. The GABA content of type 1 brown rice (not soaked) was 20.76 µg/g and it ranged from 155.03 to 284.60 µg/g after being soaked in solutions with different pH (Table 1). The GABA content of type 2 brown rice (not soaked) was 16.96 µg/g and it ranged from 40.84 to 227.38 µg/g after being soaked. The highest value of GABA content for type 1 brown rice (284.60 µg/ g) was found in samples soaked in water at pH 7, while the production of GABA was lower at pH 5 and pH 6 with respective values of 155.3 µg and 216.85 µg. Similarly, the highest value of GABA content for type 2 brown rice (227.38 µg/ g) was observed in samples soaked in water at pH 7, while the production of GABA was lower at pH 5 and pH 6 with respective values of 40.84 µg and 183.02 µg. GABA contents were significantly different based on pH levels (p<0.05), but also based on the type of brown rice.

The results showed that higher GABA content was obtained with a soaking solution at pH 7. These results were similar to those reported by Zhang *et al.* (2014) who suggested that soaking Indica and Japonica germinated brown rice at 30°C in distilled water with pH 7 resulted in the highest GABA. However, Sunte *et al.* (2007) found that Thai Hommali 105 rice soaked in a pH 5 buffer solution had the highest GABA content, while Watchraparpaiboon *et al.* (2010) measured the highest GABA content in Thai Khao Dawk Mali 105 and Chainat 1 rices when soaked in a pH 6 water, and Thai Sangyod Muang Phatthalung rice soaked in a pH 3 water for 36 hours (Banchuen *et al.,* 2009). Another study on Thai Phitsanulok 2 rice soaked at 33°C for 300 minutes in a pH 5.7 water provided the highest GABA content (Singh *et al.,* 2017). The increase of GABA content during soaking is due to the action of glutamate decarboxylase (GAD) that gradually increases during water soaking and transforms glutamate into carbon dioxide and GABA (Komatsuzaki *et al.*, 2007). During germination, biomolecules, such as lipids and proteins, increase. Soaking water acts as a hydrolytic enzyme to digest the rice seed proteins and lipids, causing an increase in permeability that allows proteins and lipids to migrate into the rice seed (Raj & Singaravadival, 1979).

Table 3. GABA content of brown rice incubated in aerobic and anaerobic conditions at different temperatures

Incubation temperature (°C)	*GABA content (µg/g)*	
	Aerobic incubation	*Anaerobic incubation*
Type 1 brown rice		
Brown rice (control)	20.76±0.73^{e}	20.76±0.73^{e}
30	536.62±2.25^{c}	494.31±14.20^{c}
35	691.88±2.30^{a}	590.50±5.95^{b}
40	597.93±12.92^{b}	508.87±10.63^{c}
Type 2 brown rice		
Brown rice (control)	16.96±2.56^{e}	16.96±2.56^{e}
30	511.11±5.02^{d}	464.17±5.13^{d}
35	596.48±6.53^{b}	557.67±11.52^{b}
40	542.32±1.86^{c}	462.98±9.97^{d}

Values represent means±*SEM* from 3 different experiments.
^{abcd} Different letters indicate significant differences between control and treatment at p<0.05.

Moreover, soaking could lead suspension cells to become adapted to water stress. Such stress may also contribute to the accumulation of GABA by reducing the oxidation of succinic semialdehyde to succinate. However, GABA accumulation in germinated brown rice at different pH levels of soaking solution is dependent on the variety and origin of rice.

Effect of incubation duration on GABA content in rice germination

As grains are being soaked, imbibition begins, respiration is accelerated, which stimulates the metabolism of amino acids, resulting in the formation of enzyme systems. GABA synthesis is usually initiated because of the activation of GAD enzyme during the soaking process, and enzyme activity increases with germination time (Karladee & Suriyong, 2012). In our study, the rice germination in aerobic incubation gave higher GABA content than anaerobic incubation in both types of brown rice and for all times of incubation. The germination duration also clearly influenced GABA content. The GABA content of type 1 brown rice had increased a lot after 18 hours, and it reached its highest value after 30 hours of incubation at 30°C (538.19 µg/g), which was 25.92 times higher than that of the control (no incubation, 20.76 µg/g) in aerobic condition. The GABA content of type 2 brown rice had also increased a lot after 18 hours and reached the highest value after 30 hours of incubation at 30°C (478.44 µg/g), which was 28.21 times higher than that of the control (no incubation, 16.96 µg/g) in aerobic condition. The results were similar in anaerobic condition for both types of Mang Buk brown rice. The results also showed that GABA content of type 1 brown rice was higher than type 2 in both conditions of incubation and for all times of incubation.

Previous studies have also shown that incubation conditions and duration have a direct impact on the metabolism of glutamic acid leading to the alteration of GABA content in sprouted brown rice. Karladee & Suriyong (2012) reported that GABA content reached its highest level of 17.87 mg/100 g of dry matter after 24 hours of incubation in 21 Thailand rice varieties (11 purple rice landraces and 10 modern white rice varieties). Patil & Khan (2011) also reported that Korean giant embryo brown rice (Keunnunbyeo) incubated for 21 hours had the highest GABA content. Similar result was also recently found by Zhang *et al.* (2022), who showed that Chinese brown rice germinated for 24 hours with pulsed light exposure gave the highest GABA content. However, working on three varieties of Southern Thai brown rice, Banchuen *et al.* (2010) suggested that the optimum conditions for producing the highest GABA content were to germinate them in a closed vessel for 36 hours for Sangyod Phatthalung rice and Chiang Phatthalung rice, and for 48 hours for Niaw Dam Peak Dam rice. GABA content was highest after 72 hours of germination for Chinese red rice (Ding *et al.*, 2018) and Indica rice (MTU 1010 and KNM 118) (Mohmmed *et al.,* 2021), or after 40 hours at 35°C for high yielding variety rice (cv. Jhelum) (Hussain *et al.*, 2020) and Brazilian red rice (Müller *et al.*, 2021). Taken together, we think genetic differences among rice varieties regulate the synthesis of GABA in grains, as genetic diversity is a basic prerequisite for successful exploitation of desirable traits through plant breeding (Hussain *et al.*, 2020).

Effect of incubation temperature on GABA content

Based on the results obtained from the changes in environment pH and incubation time, we continued to monitor GABA production during MangBuk rice germination based on incubation temperature. The effect of different

Table 4. Current reports (last fifteen years) about the optimal germination conditions for GABA enrichment in brown rice

Type of rice	*Optimal germination conditions for GABA enrichment*					*References*
	Temperature and times of soaking	*pH of soaking solution*	*Temperature of germination*	*Times of germination*	*Highest GABA content (mg/ 100g)*	
Thai Hommali 105 rice		pH 5			21.93	Sunte *et al.*, 2007
Thai Sangyod Muang Phatthalung rice		pH 3		36h	44.53	Banchuen *et al.*, 2009
Thai Khao Dawk Mali 105 and Chainat 1 rice	35°C for 24h	pH 6			16.48 for Khao Dawk Mali 105 and 14.50 for Chainat 1	Watchraparpaiboon *et al.*, 2010
Thai Sangyod Phatthalung rice and Chiang Phatthalung rice		pH 3		36h in closed vessel	44.53 for Sangyod Phatthalung and 29.25 for Chiang Phatthalung	Banchuen *et al.*, 2010
Thai Niaw Dam Peak Dam rice				48h in closed vessel	20.92	Banchuen *et al.*, 2010
Thai red Jasmine brown rice	35°C for 24h				41.02	Wichamanee & Teerarat, 2012
21 Thai rice varieties (11 landraces purple rice and 10 modern white varieties)				24h	13.65 - 23.6	Karladee & Suriyong, 2012
Japonica brown rice	3h		35°C	21h	24.9	Komatsuzaki *et al.*, 2007; Cung *et al.*, 2013.
Indica and Japonica brown rice	30°C	pH 7	35°C	36h	131 for Indica rice and 138 for Japonica rice	Zhang *et al.*, 2014
Chinese Dongnong 419 rice	30°C for 12h		25°C	40h	28.14	Cao et al., 2015
Thai Phitsanulok 2 rice	33°C for 300 min	pH 5.7			18.67	Singh *et al.*, 2017
Chinese red rice				72h	44.8	Ding *et al.*, 2018
Indica High yielding variety rice (cv. Jhelum)	5.76 h		35°C.	40h	48.18	Hussain *et al.*, 2020
Indica MTU 1010 and KNM 118 rice	28±2°C for 12h			72h	98.53 for MTU 1010 and 71.77 for KNM 118	Mohmmed *et al.*, 2021
Indonesian brown rice (var. Inpari 43)	120h			24h	126.55	Munarko *et al.*, 2021
Vietnamese MangBuk soft rice and MangBuk hard rice	30°C for 12h	7	35°C	30 hours in aerobic condition	69.188 for soft rice and 59.648 for hard rice	This study

incubation temperatures in aerobic and anaerobic conditions on GABA content are shown in Table 3.

The GABA content was highest at 35°C with both types of rice in both conditions of incubation. However, the GABA content of samples in aerobic condition was higher than that in anaerobic condition, and the GABA content in type 1 germinated brown rice was higher than in type 2 germinated brown rice. The highest GABA content of type 1 brown rice was observed at 35°C of incubation in aerobic condition (691.88 µg/g), while that of type 2 brown rice was 596.48 µg/g under the same conditions. The GABA contents of non-incubated brown rice were measured at 20.76 µg/g and 16.96 µg/g for type 1 brown rice and type 2 brown rice, respectively. After 30 minutes of incubation at 35°C, the GABA contents increased by 33.33 to 35.17 times compared to the initial contents. Therefore, 35°C was the best incubation temperature to increase GABA content during germination.

A similar result was reported by Wichamanee & Teerarat (2012) with a GABA content of 41.02 mg/100 g in red rice grain after soaking at 35°C, as well as Watchraparpaiboon *et al.* (2010) who reported that GABA contents of brown Thai rice were the highest after soaking rice grains in water at 35°C for 24 hours. Komatsuzaki *et al.* (2007) also soaked brown rice at 35°C to make GABA content reached 24.9 mg/100 g, higher than the conventional method (10.1 mg/100 g). Thus, Cung *et al.* (2013) suggested that the optimal incubation temperature of germinated rice should be 35°C. Zhang *et al.* (2014) reported that the highest GABA content was obtained by a germination at 35°C for 36 hours. However, the maximum GABA synthesised during germination was recorded in Indica Jhelum rice and Indica Tangdhar Zag rice after 5.76 hours of soaking and 40 hours of germination at 35°C (Hussain *et al.,* 2020), and in Indonesian Brown Rice (var. Inpari 43) after 120 hours of soaking and 24 hours of germination (Munarko *et al.,* 2021). Therefore, we believe that different rice varieties of different origins need different soaking temperatures, durations, and germination conditions to reach their highest GABA content (Table 4).

CONCLUSION

Germination conditions modified the content of biologically active compounds in MangBuk soft and hard rice varieties. GABA was synthesised during germination based on three factors: time of incubation, temperature of incubation, and pH of solution. The GABA content reached its highest value at 691.88 µg/g for type 1 rice and 596.48 µg/g for type 2 rice when MangBuk brown rice of Vietnam was soaked in a pH 7 water at 30°C for 12 hours, and then continually incubated at 35°C for 30 more hours in aerobic condition. These results showed that germination is an important process to preserve brown rice bioactive compounds. We also revealed, for the first time, the effectiveness of optimal conditions for maximising GABA accumulation in MangBuk brown rice in Vietnam.

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Authors' contributions

HTNT, experiments realisation and data analysis; PPH, principal investigator, study conceptualisation and design, data analysis and interpretation, manuscript draft preparation; TMDN, data analysis and interpretation, manuscript draft preparation and review.

Conflict of interest

The authors declare no conflict of interest.

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Cathelicidin LL-37 level in presence and absence of vitamin D in cultured macrophages isolated from elderly women

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ABSTRACT

Introduction: Vitamin D deficiency and frequent infections are the two common worldwide phenomenon among elderly. Recent studies have demonstrated that vitamin D regulates the expression of specific endogenous antimicrobial peptides like cathelicidin LL-37 of macrophages and neutrophils, which is active against a broad spectrum of infectious agents. Therefore, the objective of the present study was to determine the level of cathelicidin LL-37 in macrophages of elderly women (classified according to serum 25(OH)D level) after exposure to *Vibrio cholera* infection and to find out the effect of $1,25(OH)_2D$ added *in vitro*. **Methods:** This study was conducted among 40 randomly selected rural elderly women aged between 60 to 70 years of age. Their vitamin D status was assessed by the estimation of serum 25(OH)D and classified into three groups viz. sufficient (14 members), insufficient (13 members), and deficient (13 members). Later, their peripheral blood mononuclear cells (PBMC) were isolated and cultured from fresh blood. $1,25(OH)_2D$ supplementation was given selectively at a dose of 10×10^{-8} M for 72 hours in the culture media; then exposed to infection and screened according to the objectives of this study. **Results:** Macrophages in all groups, except vitamin D deficient group, responded significantly in terms of LL-37 release during exposure to *Vibrio cholera* infection. Considering *in vitro* $1,25(OH)_2D$, supplementation responded significantly ($p<0.05$) in all three groups. **Conclusion:** Vitamin D can be used as a prophylaxis to enhance cathelicidin LL-37 release for all three groups as in the present study.

Keywords: cathelicidin LL-37 activity, elderly women, macrophages, peripheral blood mononuclear cells, vitamin D

INTRODUCTION

The world population of 60 years and above is expected to increase from 962 million to 2.1 billion in the year 2050, which will produce major difficulties in healthcare systems throughout the world (World Population Ageing, 2017). Infections and septicaemia are common among the elderly (Nasa, Juneja & Singh, 2012). Vitamin D deficiency is also a worldwide phenomenon among the elderly (Pan *et al.*, 2016). Vitamin

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D has a vital role in the body's defence mechanism towards infection by promoting the roles of macrophages and monocytes, which are important in pathogenesis (Yamshchikow *et al.*, 2009). Adjunctive treatment of vitamin D against different infections has been reported (Soeharto *et al.*, 2019).

One of the major components of vitamin D-mediated antimicrobial activity is through the production of peptides. It has already been proven that vitamin D stimulates the expression of potent antimicrobial peptides, such as cathelicidin LL-37 (Bartley *et al.*, 2010) and β defensin 2 (Bartley *et al.*, 2010). The neutrophils, monocytes, natural killer (NK) cells, and epithelial cells lining of the respiratory tract exerts these peptide synthesis with the help of vitamin D (Ginde, Mansbach & Camargo, 2009). Various research works from different parts of the world have shown that macrophages, lymphocytes, and monocytes have vitamin D receptors (VDRs) that, with 25(OH)D stimulation, increase the expression of these antimicrobial peptides (Schwalfenberg, 2011, Jeng*et al.*, 2009, Bikle, 2008).

Cathelicidin LL-37, an endogenous antimicrobial peptide, is active against a broad spectrum of infectious agents including gram negative and positive bacteria, fungi, and mycobacteria (Dürr, Sudheendra & Ramamoorthy, 2006). It is highly expressed at barrier sites including respiratory and colonic epithelium, saliva, and skin; thus provides an important first line defence mechanism for the innate immune system to respond against infectious insults (Liu *et al.*, 2006). *In vitro* $1,25(OH)_2D$ treatment of infected cultured macrophages can enhance the expression of cathelicidin LL-37 (Liu *et al.*, 2006). Stimulated macrophages cultured in vitamin D deficient sera are unable to up-regulate LL-37 and effectively kill *Myobacteriumtuberculosis* (Mtb) (Liu*et al.*, 2006). However, the addition of 25(OH)D in the media up-regulates the production of LL-37 and restores effective killing of Mtb, suggesting that vitamin D has an important role in the production of anti-microbial peptides, which is important for innate immunity (Liu *et al.*, 2006). On the other hand, despite playing such a crucial role (antimicrobial and immune benefits), this antimicrobial peptide also contributes to the host's defence through wound repair (Hiemstra *et al.*, 2007) and clearance of bacteria at various barrier sites (White, 2010).

There is a paucity of study regarding the association of vitamin D deficiency and human cathelicidin LL-37 activity against infection among macrophages isolated from adult above 60 years of age (Liu *et al.*, 2006, White, 2010, Yuk *et al.*, 2009, Martineau *et al.*, 2007). Hence, this study was planned as no such study has been conducted so far among elderly women. The objectives of this study were (i) to determine the level of cathelicidin LL-37 in macrophages of elderly women classified according to serum 25(OH)D level, and (ii) to evaluate the effect of $1,25(OH)_2D$, added *in vitro*, on cathelicidin LL-37 level in macrophages of the target population.

MATERIALS AND METHODS

Sampling design

The present study was a small part of an original research work already published (Ghosh *et al.,* 2020), where the actual sample size was 236. The sample size was calculated based on a previous prevalence of Vitamin D deficiency at 91.2% (Kota *et al.,* 2011); and using the formula $n=(Z\ (1-\infty/2))^2pq/L^2$; where L is allowable error, which was taken as 5% of p, and $Z\ (1-\infty/2)$ is the standard normal deviate at 95% confidence limit, which was 1.96. The calculated sample size came to be 145. Since multistage

random sampling was adopted, it was multiplied by 1.5 (design effect), which came to 217.5. An additional 10% was added to compensate for dropout, which was then calculated to be 239. Finally, 236 participants were included. From that 236 samples, one sub-sampling (with proper randomisation technique) was done to observe the cathelicidin LL-37 activity of cultured macrophages of the target population. Hence, the sample size was 40.

These 40 elderly women were selected randomly from those 236 samples previously screened, who were residing at 80 different villages of Amdanga block, 24 Parganas North, West Bengal, India, during April 2014 to August 2018. Mean age of the target population was 62.5±4.2 years. They were classified into three groups: vitamin D sufficient group (14 members), vitamin D insufficient group (13 members), and vitamin D deficient group (13 members) as per their serum 25(OH)D levels. Deficiency, insufficiency, and sufficiency of vitamin D were defined as ≤20, 21–29, and ≥30 ng/ml of serum 25(OH)D in human blood, respectively (National Institute of Health, 2020).

In the final stage, their peripheral blood mononuclear cells (PBMC) were isolated from fresh blood (4ml) and were screened. Elderly women having a previous history of thyroid dysfunction, on hormonal replacement therapy, amenorrhoea due to any pathological cause or surgery, on vitamin D supplementation, physically or mentally challenged, and non-cooperative in nature were excluded from the study. Elderly women having fever in the last 20 days, having high total WBC count and high C-reactive protein level were excluded from the study. Ethical clearance was obtained from the Ethical Committee of All India Institute of Hygiene and Public Health (AIIH&PH), Kolkata. Informed written consent was obtained prior to the study.

Isolation and culture of human macrophages

Peripheral blood mononuclear cells (PBMC) were isolated from heparinised blood (4ml) of healthy older adult women volunteers by density gradient centrifugation with Ficoll-Paque (Tyurina*et al.*, 2007). Isolated cells were washed twice in phosphate-buffered saline (PBS) and were resuspended in medium RPMI 1640 (HIMEDIA), supplemented with 10% Fetal Calf Serum and Macrophage Cell Stimulating Factor (MCSF) at 2ng/ml concentration. Finally, cells were added to adherent six-well plates at a density of 2×10^6 cells per well. After incubation for 48 hours, at 37°C and 5% CO_2 environment, the non-adherent cells were removed by repeated vigorous washings. Selected cell culture was then supplemented with $1,25(OH)_2D$ at a dose of 10×10^{-8} M for 72 hours. The dose was standardised and referred to previously (Dalton, Shertzer & Puga, 1999). After completion of seven days culture, isolated cells were infected with *V. cholerae* (1:40) and were kept at 37°C for 120 minutes. Uninfected cells without $1,25(OH)_2D$ supplementation, infected cells without $1,25(OH)_2D$ supplementation, and infected cells with $1,25(OH)_2D$ supplementation were prepared. For *in vitro* vitamin D supplementation, active form of vitamin D ($1,25(OH)_2D$) was used for direct acceptance of macrophages during exposure to infection and better induction for LL-37 release.

Estimation of serum 25(OH)D level and cathelicidin LL-37 level

Serum 25(OH)D and cellular LL-37 levels were measured by enzymatic immunoassay (LL-37(Human) ELISA kit., 2018; Holick, 2007). Precision of

the estimation was determined by intra-assay and inter-assay variabilities. Deficiency, insufficiency, and sufficiency of vitamin D were defined as ≤20 ng/ml, 21–29 ng/ml, and ≥30 ng/ml of serum 25(OH)D in human blood, respectively (National Institute of Health, 2020).

Statistical analysis

In LL-37 level assay, continuous data were tested for normal distribution and significant Kolmogorov-Smirnov tests were observed. Friedman analysis of variance (ANOVA) was used to compare the repeated measures of the same groups. Kruskal-Wallis test was used to compare three different groups. The Graph pad prism 7.04 and IBM SPSS Statistics version 20.0 (IBM Corp, Armonk, New York, USA) were used for statistical analysis. P-value of less than 0.05 was considered as statistically significant.

RESULTS

In the vitamin D sufficient group, cathelicidin LL-37 levels in isolated macrophages were significantly increased (p<0.05) after exposure to *V. cholerae*, which was further increased significantly (p<0.05) on *in vitro* $1,25(OH)_2D$ supplementation (Table 1).

Cathelicidin LL-37 levels in isolated macrophages of vitamin D insufficient group were increased significantly after infection (p<0.05), which were further increased significantly owing to *in vitro* $1,25(OH)_2D$ supplementation (p<0.05). On the other hand, cathelicidin LL-37 levels from macrophages of vitamin D deficient group, though not increased significantly after exposure to infection, did increase significantly after *in vitro* supplementation of $1,25(OH)_2D$ (p<0.05).

No significant differences were observed in the cathelicidin LL-37 levels in isolated macrophages of the three groups viz. vitamin D sufficient, insufficient, and deficient; without *V. cholerae* infection, with *V. cholerae* infection, and with *V. cholerae* infection accompanied by $1,25(OH)_2D$ supplementation.

DISCUSSION

Recent discovery of the immuno-modulatory role of vitamin D, specifically its induction of antimicrobial peptide gene expression, explains the 'antibiotic' effect of vitamin D that has greatly renewed interest in the ability of vitamin D to improve immune functions (Wang *et al.*, 2004). Up-regulation of antimicrobial peptide gene expression because of

Table 1. Cathelicidin LL-37 level in cultured human macrophages of elderly women according to 25(OH)D levels with or without exposure to *Vibrio cholerae* infection (n=40)

Cathelicidin LL-37 (ng/ml)	*Vitamin D deficient group Median (IQR) (ng/ml)*	*Vitamin D insufficient group Median (IQR) (ng/ml)*	*Vitamin D sufficient group Median (IQR) (ng/ml)*	*Kruskal Wallis test*
Without infection	78.22(34.71)	75.01(56.32)	67.20(45.37)	2.03
Infection	77.58(56.48)	138.94(91.39)	99.24(89.78)	4.24
Infection with $1,25(OH)_2D$ treatment	99.83(38.63)	192.00(123.25)	111.12(103.10)	5.13
Friedman ANOVA	6.61*	14.00*	17.71*	

*p<0.05

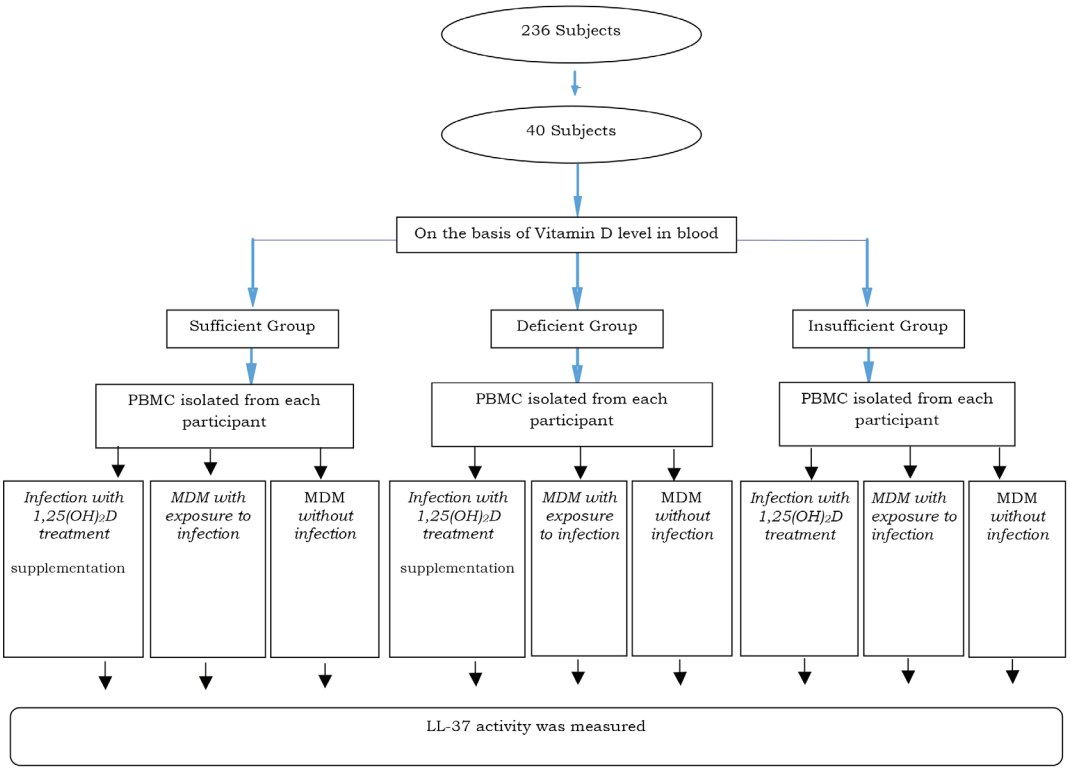

Figure 1. Details of work flow

$1,25(OH)_2D$ supplementation was discovered more than a decade ago (Liu *et al.*, 2007, Martineau *et al.*, 2007). It was also demonstrated that *in vitro* TLR 2/1 (Toll Like Receptor) signalling by a synthetic 19-kD *M. tuberculosis*–derived lipopeptide enhanced the antimicrobial capacity of monocytes via a vitamin D and VDR-dependent pathway (Liu *et al.*, 2007). Several reports have shown that such incidence was involved in the induction of CAMP gene (LL-37 gene) and its protein expression (Liu *et al.*, 2007, Martineau *et al.*, 2007, Nursyam, Amin & Rumende, 2006).

While these reports prove that vitamin D boosts anti-mycobacterial immunity *in vitro,* there are also many *in vivo* reports where vitamin D supplementation trials were conducted to find out its immunomodulatory role in CAMP induction (Gombart, Borregaard & Koeffler, 2005; Nallelyl *et al.*, 2014; Adams *et al.*, 2009). Most immune cells in the human body have in-built VDR expressions that initiate production of antimicrobial peptides after receiving stimulation from 25(OH)D (Schwalfenberg *et al.,* 2011). Epidemiological studies in the United States revealed a positive relationship between serum 25(OH)D and cathelicidin LL-37 levels among acute septicaemia patients (Jeng *et al.*, 2009; Routsias *et al.*, 2010). One interesting study by Adams *et al.* (2009) showed that during vitamin D insufficient conditions, cultured monocytes showed increased expression of the vitamin D-activating enzyme CYP27b1, but decreased expression of cathelicidin LL-37 antimicrobial peptide (hCAP) mRNA. Again, vitamin D

supplementation increased hCAP mRNA expression significantly (Adams *et al.,* 2009).

Our study revealed significant increase of cathelicidin LL-37 levels in cultured macrophages isolated from elderly women having sufficient and insufficient serum 25(OH)D levels when infected with *V. cholerae*. This was not observed in the serum 25(OH)D deficient group, which is in accordance with earlier reports (Jeng *et al.*, 2009; Liu *et al.*, 2006; Adams *et al.,* 2009). This indicates that cathelicidin LL-37 expression can be down-regulated owing to very low levels of serum 25(OH)D (Jeng *et al.*, 2009, Adams *et al.,* 2009), that is associated with higher susceptibility to nosocomial infections like pneumonia, sepsis, and central line infections (Bikle, 2008).

Though the cathelicidin LL-37 levels in macrophages isolated from serum 25(OH)D deficient group did not significantly increase after exposure to *V. cholerae* infection, *in vitro* supplementation of $1,25(OH)_2D$ increased the levels significantly ($p>0.05$). Similar observations were made in earlier report as well (Adams *et al.*, 2009). Thus, vitamin D plays a key role in innate immunity by maintaining localised production of anti-microbial LL-37 following TLR activation of monocytes/macrophages (Adams *et al.*, 2009).

Similar work was published demonstrating that $1,25(OH)_2D$ treatment of macrophages infected with *M. tuberculosis in vitro* enhanced the production of an endogenous anti-microbial peptide, cathelicidin LL-37 and ameliorate the killing of the microorganisms (Liu *et al.*, 2007). In another study, it was observed that most of the diabetes mellitus 2 (DM2) patients with low VDR had low antimicrobial peptides (AMPs) expression, but when monocyte derived macrophages (MDMs) from patients having DM2 and having low VDR expression were supplemented with $1,25(OH)_2D$, MDMs eliminated more *M. tuberculosis* (Nallelyl *et al.*, 2014). The authors suggested the use of vitamin D as a prophylaxis for tuberculosis in high DM2 endemic countries (Nallelyl *et al.*, 2014). According to Hacıhamdioğlu *et al.* (2016), children with vitamin D insufficiency may not be able to increase their urine cathelicidin LL-37 levels during urinary tract infection caused by *Escherichia coli* (Hacıhamdioğlu *et al.,* 2016). Again, according to Adams *et al.* (2009), the ability of human macrophages to induce cathelicidin LL-37 level in response to TLR-activation is directly proportional to serum 25(OH) D status; thus it can be enhanced in vitamin D insufficient patients with supplementary vitamin D.

CONCLUSION

The present study revealed that vitamin D status has a strong influence on cathelicidin LL-37 level of macrophages and subsequent protection against infection in elderly women, but up to the stage of insufficiency. Further study may demonstrate the efficacy of higher $1,25(OH)_2D$ supplementation doses in elevating the cathelicidin LL-37 level in macrophages among elderly with vitamin D deficient status.

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Authors' contributions

JG, conducted the study, prepared the draft of the manuscript and reviewed the manuscript; also led the data collection and did all the biochemical, microbiological experiments; ANC, conceptualised, designed and conducted the study, reviewed

the manuscript, reviewed the data analysis and interpretation, and assisted in drafting of the manuscript; IS, reviewed the manuscript, and reviewed the data analysis and interpretation; DC, conceptualised and designed the study, reviewed the manuscript, reviewed the data analysis and interpretation, and assisted in drafting of the manuscript.

Conflict of interest

There are no conflicts of interest.

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Models and theories to support health promotion programmes for overweight and obese adults: A scoping review

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ABSTRACT

Introduction: The increasing prevalence of overweight and obesity has been alarming. One approach to address this issue is to implement health promotion programmes. Despite the many health promotion and health intervention programmes held, there is a lack of evidence showing the application of theories and models. This scoping review aimed to explore existing literature and synthesise findings based on models and theories used to support health promotion programmes for overweight and obese adults. **Methods:** This review was guided by the Arksey and O'Malley framework. Online databases, such as Ovid, PubMed, Scopus, and Web of Science, were used to search for relevant articles using suitable keywords, from January 2015 until December 2021. Articles were written in English and Malay, and the study subjects were adults aged 18-59 years old. Two reviewers independently screened the articles, and the extracted information were tabulated after analysis. **Results:** A total of 13 different theories and models were found in the 27 articles selected. Many studies reported using the Transtheoretical Model, Social Cognitive Theory, and Health Belief Model in health promotion programmes for overweight and obese adults. Most constructs and components focused on changing health-related behaviours starting with the individual, such as self-efficacy and readiness to change. Majority of the articles showed an improvement in health-related behaviours and had great potential for future studies. **Conclusion:** When conducting health promotion programmes for overweight and obese adults, researchers should consider models and theories with constructs and components to ensure consistent improvement and potentially significant impacts on health-related outcomes.

Keywords: adult, health promotion, model, obesity, theory

INTRODUCTION

Overweight and obesity are significant public health issues. The prevalence of overweight and obesity has dramatically increased globally. In 2016, it was found that 39% of adults aged 18 years and

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above among the world's population were overweight, while 13% were obese (WHO, 2021). These are alarming figures because the risk of mortality due to overweight or obesity is higher than being underweight. Overweight and obesity are public health concerns because they increase the risk of chronic diseases such as high blood pressure (Aniza *et al.*, 2015), stress and depression (Rosengren *et al.*, 2015), anxiety (Abdollahi & Abu Talib, 2015), diabetes, cancer, and cardiovascular diseases (Moghaddam, Woodward & Huxley, 2007). Indirectly, obesity-related treatment costs have risen (Atella *et al.*, 2015; Correia & Laviano, 2018; Marcellusi *et al.*, 2016), as has the investment in intervention programmes at the individual, environment, and policy levels (Flegal *et al.*, 2010). Nevertheless, this epidemic can still be prevented (De Lorenzo *et al.*, 2020).

One of the strategies to address overweight and obesity issues is through health promotion programmes. The World Health Organization (WHO) defines health promotion as a process of enabling people to control and improve their health. Programmes, such as obesity interventions, are also part of health promotion programmes that include various social and environmental interventions to improve weight management and quality of life, and mainly to prevent diseases. Hence, implementing health promotion programmes is one of the approaches that is scientifically proven to improve health among individuals and communities (Khodaveisi *et al.*, 2017). Past studies have shown that theories and models have a beneficial positive impact on health promotion programmes for overweight and obese adults (Martin *et al.*, 2015; Martinez *et al.,* 2017), whereby they can further assist in reducing morbidity and mortality by changing the behaviours of the population (Crosby & Noar, 2010). Therefore, a programme based on theories such as health promotion or behaviour modification will be more efficient and effective (Fertman & Allensworth, 2016; Sanaeinasab *et al.*, 2020).

However, there is a limited body of literature that collects information about models and theories, as well as their components or constructs, which have been used in health promotion programmes for overweight and obese adults. For this reason, this scoping review was conducted to systematically map the existing literature on models and theories that have been used in health promotion programmes for overweight and obese adults. It is also an effort to map out the constructs and components, which gives insight into the importance of using theories and models in health promotion programmes.

MATERIALS AND METHODS

Study design

A scoping review was conducted to map the available literature and key concepts informing the models and theories used to support health promotion programmes for overweight and obese adults. The method was based on the Arksey & O'Malley (2005) framework for scoping review. The approach to searching, screening, and reporting was modified and utilised as suggested by Levac, Colquhoun & O'Brien (2010) and the Joanna Briggs Institute (Peters *et al.*, 2020). The PRISMA Extension for Scoping Reviews (PRISMA-ScR) was used to inform this review.

Identifying the research questions

Factors or descriptive characteristics that were relevant to the topic and how the research was conducted were suggested to be included while developing the research questions. (Peters *et al.,* 2020; Munn *et al.,* 2018). Therefore,

research questions were identified based on descriptive characteristics relevant to the topic. In the meantime, a preliminary review was conducted to understand the importance and significant impact of using theories and models in health promotion programmes. Thus, research questions were generated as follows:

i) What are the characteristics and health measurements of a study involving theories and models in a health promotion programme for overweight and obese adults?
ii) What are the theories and models, as well as the constructs or components found in the studies included?
iii) What is known from the literature about theories and models used in health promotion programmes for overweight and obese adults that resulted in improved and potentially significant health-related outcomes?

Identification of studies

Articles published from January 2015 until December 2021 that were written in English or Malay and described the theories and models used in health promotion programmes for overweight and obese adults aged 18-59 years old were selected. Meanwhile, articles involving children, adolescents, elderly, pregnant and menopausal women, patients using medicine, supplement, or herbal treatments, as well as studies with any specific chronic and non-communicable diseases, such as heart attack, diabetes, cancer, mental illness, mental disability and many more, were excluded. Review articles, protocol articles, qualitative and pilot studies were also excluded in this review.

To identify relevant documents, the following bibliographic databases were searched – Ovid, PubMed, Scopus, and Web of Science (WOS) – by utilising the keywords outlined in Table 1. Reference lists of retrieved articles were also checked to identify additional articles of interest. The key terms in the search strategy were theor*, model*, obes*, overweight, body mass index, fat*, adult*, health program*, health campaign*, health education*, health intervention*, wellness program*, and adult*. Results were sorted using the Mendeley software, and duplicates were omitted.

Table 1. List of keywords and synonyms generated as search terms

Theory	*Obesity*	*Health promotion*	*Adult*
Model	Overweight	Health programme	
	Obese	Health campaign	
	Body mass index	Health education	
	Fat	Health intervention	
		Wellness programme	

Study selection

Two steps of screening were completed to select the related articles, and the Mendeley software was used to assist in this process. In the first step, two reviewers named TS and RAT independently screened all the retrieved articles. The articles' titles, abstracts, and keywords were sorted if they met the inclusion and exclusion criteria. Selected articles that both reviewers agreed on proceeded to the second step. Upon any disagreement, the article was decided in the second step on whether to be included or omitted from this study. Articles were excluded if both reviewers agreed that they did not meet any of the study's criteria. The same process was repeated in the second step, but the whole article was screened and reviewed this time. Further discussion was held until both reviewers reached a consensus on

whether to include or exclude the article. For any inconsistency in deciding the article selection, a third reviewer helped to make the final decision. The PRISMA 2020 flow diagram (Page *et al.*, 2021) in Figure 1 was used to illustrate the study selection.

Charting the data

A standardised data-charting form to extract information from the articles that corresponded with the research questions was developed and tested by a reviewer. The form was refined and improved by other reviewers. Then, the form was finalised to ensure all the categories were appropriate and consistent throughout this process. The following variables were included such as article characteristics (authors, year of publication, continent), study characteristics (study design, study duration, and health measurement), theory and model along with the construct or component, and outcome or impact of the studies. Two reviewers independently charted the data, and the form was updated from time to time while discussing the results. Any discrepancy in extracted data was discussed further by the reviewers' team until an agreement was reached.

Collating, summarising, and reporting results

The collated data were analysed using descriptive analysis and tabulated into several forms, such as frequency and percentage. The descriptive analysis helped to identify the frequency of theories and models that have been used in health promotion programmes. In addition, study characteristics like location, study design, and setting were also presented in the results. The constructs or components of theories and models were tabulated using thematic analysis, while the outcomes or impacts were summarised in a table.

RESULTS

A total of 11,371 articles were identified from four different databases. From this sum, 26 articles were excluded due to duplication, while another 11,232 articles were removed based on the inclusion and exclusion criteria. The titles, abstracts, and keywords from 113 articles were screened, and 76 articles were discarded. After retrieving 37 articles to be screened entirely, 10 articles were excluded for several reasons, as shown in Figure 1. Therefore, a total of 27 articles were included in this scoping review.

Study findings

Characteristics and health measurements of the included studies

All 27 articles included in this study reported evidence of a health promotion programme for overweight and obese adults. A greater proportion of the studies were from North America (55.6%) and Asia (29.6%). Two studies were conducted in Australia, and one was conducted in Europe and South America. Most of the studies (44.4%) were conducted in the community setting (Arevalo & Brown, 2019; Armitage, Alganem & Norman, 2017; Tucker *et al.*, 2019; Choo & Kang, 2015; Ekúndayò *et al.*, 2020; Griffin *et al.*, 2018; Hales *et al.*, 2016; Kite *et al.*, 2018; Powers *et al.*, 2019; Schifferdecker *et al.*, 2016; Spurrier *et al.*, 2018; Vandelanotte *et al.*, 2018). In comparison, five (18.5%) were conducted in the workplace (Abdi *et al.*, 2015a; Abdi *et al.*, 2015b; Ott *et al.*, 2015; Sanaeinasab *et al.*, 2020; Silberman *et al.,* 2020) and institutional settings, respectively (Dong & Branscum, 2019; Johnson & Annesi, 2017; Romain, Horwath & Bernard, 2018; Saghafi-Asl, Aliasgharzadeh & Asghari-Jafarabadi, 2020; Wright *et al.*, 2020); three (11.1%) in a health care setting (de Menezes *et al.*, 2015; Nazari *et al.*, 2019; Winik & Bonham, 2018); and one each in a

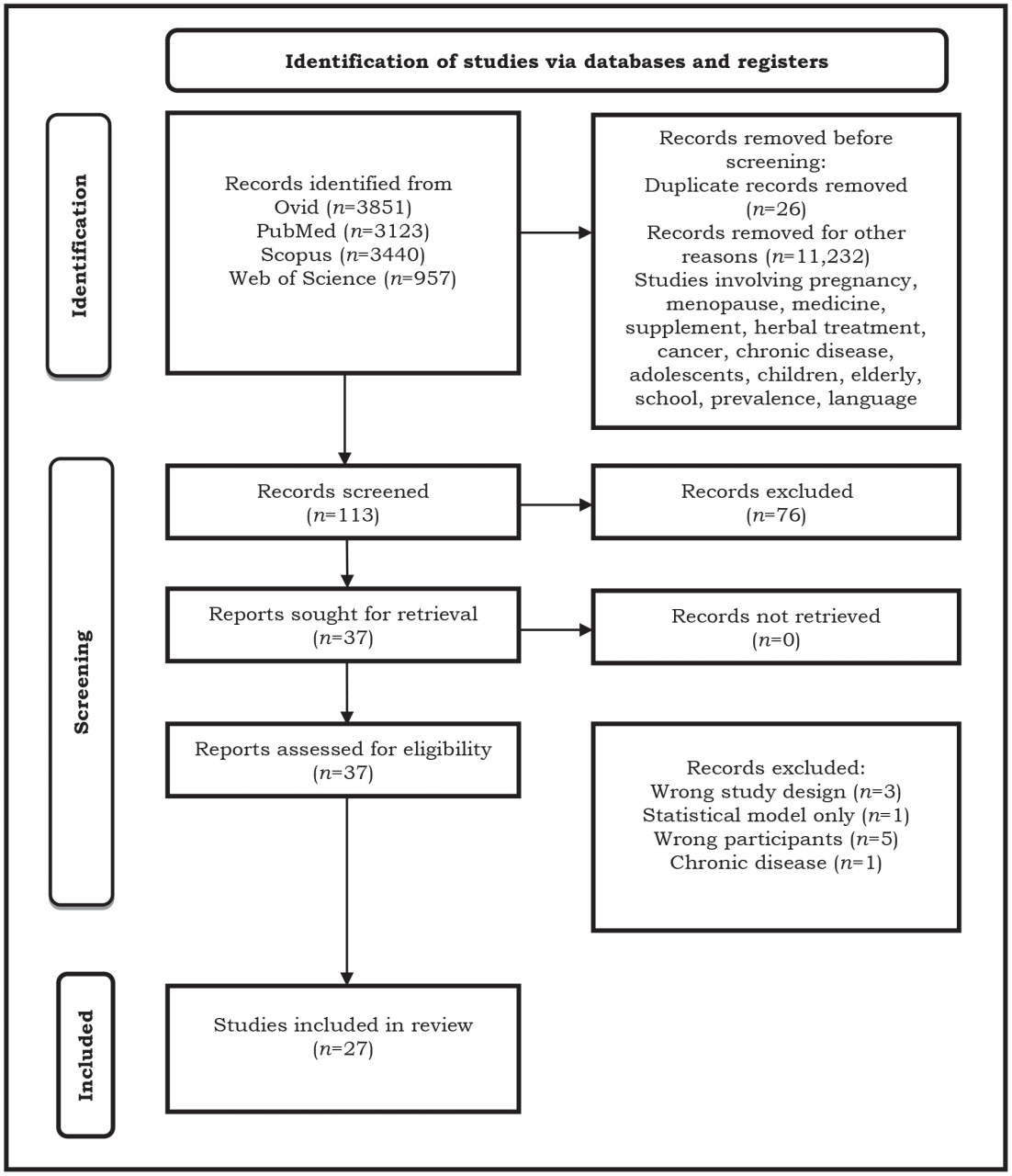

Figure 1: PRISMA 2020 flow diagram for study selection

church (Lin *et al.*, 2015) and a sports centre (Cingil & Göger, 2020).

Table 2 presents the characteristics of the included studies and health assessments reported. The included studies employed different study designs, including 17 (63.0%) experimental studies such as pre- and post-intervention (Arevalo & Brown, 2019; Tucker *et al.*, 2019; Spurrier *et al.*, 2018; Wright *et al.*, 2020); pre-test and post-test (Griffin *et al.*, 2018;

Table 2: Characteristics and health measurements of the studies

No	*Authors & publication year*	*Country/ continent*	*Setting*	*Study design*	*Study duration*	*Health measurement(s)*
1	Abdi *et al.* (2015b)	Western Iran/ Asia	Workplace	Randomised controlled trial	12 months	BMI, weight, waist circumference, and blood pressure.
2	Abdi *et al.* (2015a)	West of Iran/ Asia	Workplace	Cross-sectional	Data were collected in 2014	BMI. FANTASTIC lifestyle questionnaire.
3	Arevalo & Brown (2019)	Texas, USA/ North America	Community	Pre-post intervention	12 months	BMI and blood pressure.
4	Armitage *et al.* (2017)	Kuwait/Asia	Community	Randomised controlled trial	6 months	Weight and height. Volitional help sheet.
5	Tucker *et al.* (2019)	Florida, USA/ North America	Community	Pre-post test	3 months	Weight, height, clinical assessment such as diastolic and systolic blood pressure, and Newest Vital Sign. Health behaviour and lifestyle were assessed using Health Information Questionnaire, Health-Promoting Lifestyle Profile-II, and Health-Smart Behaviour Frequency Scale.
6	Choo & Kang, (2015)	Korea/Asia	Community	Longitudinal correlational	12 months	Weight, height, and BMI. Physical activity and lifestyle were measured by The Weight Efficacy Lifestyle (WEL) questionnaire, The Exercise Self-Efficacy (Exercise SE) Scale, and The Health-Promoting Lifestyle Profile-II (HPLP-II).
7	Cingil & Göger (2020)	Turkey/Asia	Sport centre	Quasi experimental	6 months	Height, weight, waist circumference, hip circumference, and BMI. Lifestyle was measured using Healthy Lifestyle Behaviours Scales II (HLBS II).

No	Authors & publication year	Country/ continent	Setting	Study design	Study duration	Health measurement(s)
8	de Menezes *et al.* (2015)	Brazil/South America	Primary care	Randomised controlled trial	6 months	BMI and waist circumference.
9	Dong & Branscum (2019)	Texas, USA/ North America	Institutional	Cross-sectional	Mention	Questionnaire related with health behaviour.
10	Ekúndayò *et al.* (2020)	North Omaha Nebraska, USA/North America	Community	Cross-sectional	Did not mention	Physical activity level was assessed using REACH Health Activity Assessment Questionnaire.
11	Griffin *et al.* (2018)	Alabama, USA/ North America	Community	Pre-test and post-test	12 weeks	Weight, height, and dietary intake. Physical activity level using Physical Activity Readiness Questionnaire (PAR-Q).
12	Hales *et al.* (2016)	South Carlina, USA/North America	Community	Randomised clinical trial	3 months	BMI, physical activity, and dietary intake. Physical activity level using Physical Activity Readiness Questionnaire (PAR-Q).
13	Johnson & Annesi (2017)	South-eastern, USA/North America	Institutional	Biweekly treatment	24 months	Weight, BMI, waist circumference, and dietary intake. Physical activity using Godin-Shephard Leisure-Time Physical Activity, set of questionnaires measuring self-regulation for exercise (SR-exercise), self-regulation for controlled eating (SR-eating), Exercise Self-Efficacy Scale, and Weight Efficacy Lifestyle Scale.
14	Kite *et al.* (2018)	New South Wales/ Australia	Community	Cohort	12 months	Fast-food intake. Physical activity level and online survey.

No	Authors & publication year	Country/ continent	Setting	Study design	Study duration	Health measurement(s)
15	Lin *et al.* (2015)	Baltimore, USA/North America	Church	2-arm randomised controlled trial	6 months	Weight, body composition, blood pressure, and waist circumference.
16	Nazari *et al.* (2019)	Iran/Asia	Health service centre	Cross-sectional	Data were collected from November 2017–January 2018	BMI. Physical activity level and exercise.
17	Ott *et al.* (2015)	Utah, USA/ North America	Workplace	Cross-sectional	Data were collected in Spring 2012	BMI, total cholesterol, fruit, and vegetable intake. Physical activity.
18	Powers *et al.* (2019)	Alabama, USA/ North America	Community	Pre-test and post-test	9 weeks in 2017	Healthy dietary intake. Assessment on behaviour and practice in physical activity.
19	Romain *et al.* (2018)	France/Europe	Institutional	Cross-sectional	Mention	BMI. Physical activity behaviour and scale was measured using the International Physical Activity Questionnaire (IPAQ). Tobacco intake was categorised under lifestyle.
20	Saghafi-Asl *et al.* (2020)	Iran/Asia	Institutional	Cross-sectional	Data were collected from June–September 2018	BMI.

No	Authors & publication year	Country/ continent	Setting	Study design	Study duration	Health measurement(s)
21	Sanaeinasab *et al.* (2020)	Iran / Asia	Workplace	Randomised controlled trial	3 months	BMI, fasting blood sugar, cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), and lipid levels. Physical activity level by International Physical Activity Questionnaire (IPAQ) and Theory of Planned Behaviour (TPB).
22	Schifferdecker *et al.* (2016)	New Hampshire & Vermont, USA/ North America	Community	Quasi-experimental	10 months	BMI. A 6-minute walk test to measure physical activity or exercise.
23	Silberman *et al.* (2020)	California, USA/North America	Workplace	Retrospective, observational design	12 months	BMI.
24	Spurrier *et al.* (2018)	West Virginia, USA / North America	Community	Pre-post intervention	12 weeks	BMI and nutrition intake. Physical activity was assessed by the Godin Leisure-Time Exercise Questionnaire and the Patient-Centered Assessment and Counselling for Exercise (PACE).
25	Vandelanotte *et al.* (2018)	Australia	Community	Randomised controlled trial	3 months	BMI. Measuring physical activity changes using Active Australia Survey, Godin Shephard Leisure-Time Exercise Questionnaire for non-Fitbit group and Workforce Sitting Questionnaire.
26	Winik & Bonham (2018)	Midwest, USA/ North America	Ambulatory care	Pre-test and post-test	6 months	BMI. Fitness assessment.
27	Wright *et al.* (2020)	USA/North America	University / Institutional	Pre-post design	6 weeks	BMI, waist circumference, body composition, blood pressure, dietary intake, fruit, and vegetable consumption. Exercise and sleep quality and quantity.

Powers *et al.*, 2019; Winik & Bonham, 2018); randomised controlled trials (Abdi *et al.*, 2015b; Armitage *et al.*, 2017; de Menezes *et al.*, 2015; Hales *et al.*, 2016; Lin *et al.*, 2015; Sanaeinasab *et al.*, 2020; Vandelanotte *et al.*, 2018); quasi-experimental (Cingil & Göger, 2020; Schifferdecker *et al.*, 2016); and treatment (Johnson & Annesi, 2017). The remaining 10 (37%) were observational studies, for example, cross-sectional (Abdi *et al.*, 2015a; Dong & Branscum, 2019; Ekúndayò *et al.*, 2020; Nazari *et al.*, 2019; Ott *et al.*, 2015; Romain *et al.*, 2018; Saghafi-Asl *et al.*, 2020), cohort (Kite *et al.*, 2018), longitudinal (Choo & Kang, 2015), and retrospective (Silberman *et al.*, 2020). Meanwhile, the minimum duration of the included studies was less than three months (Tucker *et al.*, 2019; Griffin *et al.*, 2018; Hales *et al.*, 2016; Powers *et al.*, 2019; Sanaeinasab *et al.*, 2020; Spurrier *et al.*, 2018; Vandelanotte *et al.*, 2018; Wright *et al.*, 2020) and the maximum was 24 months (Johnson & Annesi, 2017).

The majority of the included studies, 23 out of 27, reported anthropometric measurements [weight, height, body mass index (BMI), body fat percentage, waist circumference, hip circumference, and waist-to-hip ratio] as their outcome measurements. Meanwhile, 15 of the 27 studies used physical activity to assess the results. Several studies reported on dietary intake (29.6%), such as healthy eating, fast food intake, fruits and vegetables consumption (Griffin *et al.*, 2018; Hales *et al.*, 2016; Kite *et al.*, 2018; Ott *et al.*, 2015; Powers *et al.*, 2019; Spurrier *et al.*, 2018; Wright *et al.*, 2020), lifestyle (smoking, drinking, sleeping, health behaviour) (25.9%) (Abdi *et al.*, 2015a; Tucker *et al.*, 2019; Choo & Kang, 2015; Cingil & Göger, 2020; Dong & Branscum, 2019; Romain *et al.*, 2018; Wright *et al.*, 2020), clinical measurements (18.5%) (diastolic and systolic blood pressure) (Abdi *et al.*, 2015b; Arevalo & Brown, 2019; Tucker *et al.*, 2019; Lin *et al.*, 2015; Wright *et al.*, 2020), and biochemical (7.4%), such as blood sugar level and blood cholesterol level (Ott *et al.*, 2015; Sanaeinasab *et al.*, 2020).

Theories and models with constructs or components of health promotion programmes

Table 3 enlists the principles of the theories and models, along with the outcomes and impacts of the studies. Of the 27 included studies, the majority (25.9%) applied the Social Cognitive Theory (SCT) (Abdi *et al.*, 2015b; Choo & Kang, 2015; Griffin *et al.*, 2018; Hales *et al.*, 2016; Johnson & Annesi, 2017; Nazari *et al.*, 2019; Vandelanotte *et al.*, 2018) and The Transtheoretical Model (TTM) (Abdi *et al.*, 2015a; Armitage *et al.*, 2017; de Menezes *et al.*, 2015; Lin *et al.,* 2015; Ott *et al.,* 2015; Romain *et al.*, 2018; Silberman *et al.*, 2020) while conducting health promotion programmes for overweight and obese adults. The constructs or components found in SCT were self-efficacy, social support from family and friends, self-regulation, reinforcement, outcome expectations, outcome expectancies, and behavioural capability. Most studies used one or more TTM stages, such as pre-contemplation, contemplation, preparation, action and maintenance; only one study incorporated all four TTM pillars (de Menezes *et al.*, 2015).

Four studies (14.8%) used the Health Belief Model (HBM) (Ekúndayò *et al.*, 2020; Lin *et al.*, 2015; Saghafi-Asl *et al.*, 2020; Spurrier *et al.*, 2018), which employed constructs such as perceived susceptibility, perceived severity, perceived threat, perceived barrier, perceived benefit, cues to action, and self-efficacy. Three studies (11.1%) each applied the Health Promotion Model (HPM) (Choo & Kang, 2015; Cingil & Göger, 2020; Winik & Bonham, 2018)

Table 3: Principles of theories and models with the health-related outcomes or impacts

No.	*Authors & year*	*Theory / model*	*Principle*	*Health-related outcomes / impacts*
Improvement in health behaviours or health outcomes (*n*=15)				
1	Armitage *et al.* (2017)	TTM	A volitional help sheet was built based on TTM processes of change.	Using a volitional help sheet had a significant impact on weight loss among overweight and obese participants in a weight loss programme in Kuwait.
2	de Menezes *et al.* (2015)	TTM	Using four pillars of TTM, such as stage of change, decisional balance, self-efficacy, and processes of change.	Intervention using TTM led to a reduction in high calorie and fat food intake. Weight and body perception among participants improved significantly.
3	Abdi *et al.* (2015b)	SCT	Self-efficacy, intention, situational social support, behavioural strategy, outcome expectations, and outcome expectancies.	The study found intervention effectiveness using SCT and new communication technologies, such as improvement in weight loss.
4	Griffin *et al.* (2018)	SCT	Self-regulation or goal setting, self-efficacy, behavioural and environmental factors.	Text messaging based on SCT showed improvement in dietary and physical activity behaviour and environment, positive dietary and physical activity goal setting, and reduced body weight among participants.
5	Hales *et al.* (2016)	SCT	Self-efficacy, reinforcement, outcome expectation, self-regulation, social support, and behavioural capability.	Using the Social POD mobile app significantly reduced body weight compared to a commercial tracking app.
6	Spurrier *et al.* (2018)	HBM	Individual perceptions, modifying factors, and likeliness of action were derived from perceived susceptibility, perceived severity, perceived threat, perceived barrier, perceived benefit, and cues to action.	Significant changes in nutritional intake, physical activity, and improvement in BMI.
7	Sanaeinasab *et al.* (2020)	TPB	Attitude, subjective norms, perceived behavioural control, and intention.	Using the TPB component in the education programme significantly improved self-reported physical activity and decreased participants' BMI.

No.	Authors & year	Theory / model	Principle	Health-related outcomes / impacts
8	Wright *et al.* (2020)	TPB	Attitude, subjective social norms, and perceived behavioural control.	The efficacy helped to improve health behaviour and outcomes related to reducing and preventing obesity among the college population.
9	Cingil & Göger (2020)	HPM	Components in health promotion behaviour such as spiritual development, interpersonal relations, nutrition, physical activity, health responsibility, and stress management are derived from the Health-Promoting Lifestyle Profile II questionnaire.	Effective to develop healthy lifestyle behaviours when using training and counselling based on the Health Promotion Model.
10	Tucker *et al.* (2019)	HSET	Health motivation, health knowledge, and health responsibility, health self-efficacy, active coping styles or strategies, health self-praise.	Physical activity was increased in the pre- and post-intervention. Engagement in health-smart behaviour and health disparity populations can reduce obesity and its related diseases.
11	Powers *et al.* (2019)	SEM	Institutional, interpersonal, and individual levels.	The study found positive improvement in behavioural changes such as healthy eating motivation, vegetable intake, and shopping practices.
12	Schifferdecker *et al.* (2016)	ALCM	Goal setting, planning steps, data monitoring, and social support	Using components in the model showed improvement in weight loss and positive changes in physical activity level.
13	Choo & Kang (2015)	SCT & HPM	Self-efficacy and health-promoting behaviour.	By using a path model, the study found increasing diet and exercise self-efficacy that had an impact on increasing health-promoting behaviours. Indirectly, health-promoting behaviours affected initial weight loss.
14	Johnson & Annesi (2017)	SCT & SET	Self-regulatory skills, self-efficacy, and self-regulation.	The treatment showed significant results for weight loss and weight loss maintenance among middle-aged women. Hence, the treatment may also be effective among young adult women.
15	Vandelanotte *et al.* (2018)	TPB, SDT, SCT	Intrinsic motivation, self-efficacy, intention, self-regulatory strategy through goal setting, action planning, social support, overcoming barriers, problem solving, decision making, relapse prevention, and self-monitoring.	The effectiveness of the intervention was significantly increased when incorporating web-based computer tailored intervention with physical activity trackers.

No.	*Authors & year*	*Theory / model*	*Principle*	*Health-related outcomes / impacts*
Potential impact for future study (*n*=11)				
16	Abdi *et al.* (2015a)	TTM	Stages in TTM: -Pre-contemplation -Contemplation -Preparation -Action -Maintenance	Significant in predicting obesity with age and work experience. The study also found a significant association between obesity and work experience, marital status, number of children, and gender. More than half of the Participants were in the preparation stage. It is recommended to use behaviour change theories like TTM as the basis for health education in the future.
17	Ott *et al.* (2015)	TTM	Stages of behaviour change in TTM: -Pre-contemplation -Contemplation	Overweight and obese workers were found in the pre-contemplation or contemplation stages to change their dietary behaviour and lose weight.
18	Romain *et al.* (2018)	TTM	Processes of change.	The study found that behavioural processes are the key point to predict physical activity behaviour. Therefore, processes of change can be useful to increase the level of physical activity.
19	Nazari *et al.* (2019)	SCT	Self-efficacy, self-regulation, outcome expectation, and social support from family and friends.	Based on the difference in predictive constructs of physical activity, the results showed potential to be used in intervention to increase physical activity and health status through an active lifestyle among overweight and obese women.
20	Ekúndayò *et al.* (2020)	HBM	Perceived susceptibility, perceived benefit of taking action, self-efficacy, and cues to action.	Compared with other races, African Americans were more likely to engage in Mild-to-Moderate Physical Activity (MMPA-150). Therefore, it is suggested to use different resources in access or availability for future programmes, especially living styles in different communities.

No.	Authors & year	Theory / model	Principle	Health-related outcomes / impacts
21	Saghafi-Asl *et al.* (2020)	HBM	Behavioural intention, perceived threat, perceived benefits, perceived barriers, and cues to action, self-efficacy.	Perceived threat, perceived benefits, self-efficacy in dieting and exercise, and cues to action in exercise were found as variables that predicted behavioural intention of weight management. Future studies in health education programmes, preventive health programmes, and health intervention are recommended to integrate with HBM.
22	Arevalo & Brown (2019)	RAA	Attitudes, subjective norms, perceived behavioural control, and self-efficacy.	The RAA framework was significant in determining the intention and attendance of organised exercise among Hispanics. It is also suitable for comprehending culturally-related behaviours.
23	Dong & Branscum (2019)	RAA	Attitudes, perceived norm, perceived behavioural control, and intentions.	The RAA framework is a significant model to understand behavioural intention to use Obesity-Related Direct-to-Consumer Genetic Test (ODTCGT), attitude, perceived norms, and perceived behavioural control.
24	Kite *et al.* (2018)	HOEM	Understanding, knowledge, attitude, social norms, self-efficacy, and intentions.	This study showed that HOEM has the potential to be used as a conceptual model in social marketing campaigns such as planning and evaluation.
25	Silberman *et al.* (2020)	SDT & TTM	Intrinsic motivation and maintenance stage.	Results showed possible improvement in weight loss by using digital health interventions with one-to-one coaching. Although a small number of participants had successfully reduced their weight, the mean weight loss exceeded the 5.0% benchmark.
26	Lin *et al.* (2015)	HBM, TTM, SRT	Behavioural modification strategies such as cognitive and behavioural emotion-focused content.	Tailored text message intervention showed the potential to help obese African American adults lose weight.
Decreasing in health behaviour or health outcome (n=1)				
27	Winik & Bonham (2018)	HPM	Self-initiated reconfiguration.	The main objective of reducing overweight and obesity was not achieved; instead, the rate was increased.

and the Theory of Planned Behaviour (TPB) (Sanaeinasab *et al.*, 2020; Vandelanotte *et al.*, 2018; Wright *et al.*, 2020). Constructs found in HPM were health-promoting behaviour, self-initiated reconfiguration, and mostly derived from the Health-Promoting Lifestyle Profile-II questionnaire. As for TPB, the constructs used were intention, attitude, subjective norms, and perceived behavioural control.

Two studies (7.4%) used the Self-Determinant Theory (SDT) (Silberman *et al.*, 2020; Vandelanotte *et al.*, 2018) and Reasoned Action Approach (RAA) (Arevalo & Brown, 2019; Dong & Branscum, 2019), respectively. Both studies that used SDT only employed intrinsic motivation, while studies using RAA employed attitude, subjective norms, perceived behavioural control, perceived norms, intention, and incorporated components like self-efficacy.

Meanwhile, the rest of the theories and models were each applied in one study, such as Health Self-Empowerment Theory (HSET) (Tucker *et al.*, 2019), Social Ecological Model (SEM) (Powers *et al.*, 2019), Action Learning Collaborative Model (ALCM) (Schifferdecker *et al.*, 2016), Self-Efficacy Theory (SET) (Johnson & Annesi, 2017), Hierarchy of Effect Model (HOEM) (Kite *et al.*, 2018), and Self-Regulation Theory (SRT) (Lin *et al.*, 2015). Each had different constructs, which are listed in Table 3.

Health-related outcomes and potential impacts

Participants in 15 out of the 27 included studies showed significant improvement in BMI, healthy eating, and physical activity. Meanwhile, another 11 studies showed potential to be useful for future studies, like understanding the variables and stages of changes that can predict participants' weight management (Abdi *et al.*, 2015a; Arevalo & Brown, 2019; Dong & Branscum, 2019; Ekúndayò *et al.*, 2020; Kite *et al.*, 2018; Lin *et al.*, 2015; Nazari *et al.*, 2019; Ott *et al.*, 2015; Romain *et al.*, 2018; Saghafi-Asl *et al.*, 2020; Silberman *et al.*, 2020). Only one study reported decreasing health behaviours due to an increase in weight instead (Winik & Bonham, 2018).

All three studies that used TPB reported to have a positive outcome (Sanaeinasab *et al.*, 2020; Vandelanotte *et al.*, 2018; Wright *et al.*, 2020). Six of the seven studies that applied SCT found that the health outcomes of the participants improved (Abdi *et al.*, 2015b; Choo & Kang, 2015; Griffin *et al.*, 2018; Hales *et al.*, 2016; Johnson & Annesi, 2017; Vandelanotte *et al.*, 2018). Studies from each theory like ALCM (Schifferdecker *et al.*, 2016), HSET (Tucker *et al.*, 2019), SEM (Powers *et al.*, 2019), and SET (Johnson & Annesi, 2017) also reported to have improved health outcomes.

The use of TTM showed more potential for use in future research (Abdi *et al.*, 2015a; Lin *et al.*, 2015; Ott *et al.*, 2015; Romain *et al.*, 2018; Silberman *et al.*, 2020), while the rest resulted in increased health outcomes (Armitage *et al.*, 2017; de Menezes *et al.*, 2015). Similarities in results were also found in studies using HBM (Ekúndayò *et al.*, 2020; Lin *et al.*, 2015; Saghafi-Asl *et al.*, 2020), RAA (Arevalo & Brown, 2019; Dong & Branscum, 2019), SRT (Lin *et al.*, 2015), and HOEM (Kite *et al.*, 2018), which were shown to be more likely to be beneficial for future research.

DISCUSSION

This scoping review extracted and explored 27 articles on health promotion programmes that were based on theories and models. The first research question was to describe the characteristics and health measurements of the studies found. Majority of the studies were from North America, were conducted in

community settings, used experimental studies, were less than three months in duration, and reported health measurements using anthropometry. Based on the results, all studies from North America were located in the United States of America. The alarming prevalence of overweight and obese individuals might cause an increased number of health promotion programmes to intervene in this situation. Aside from community settings, various study settings were discovered, including workplaces, institutions, health care facilities, churches, and sport centres. Choosing a controllable setting may increase the chances of a programme's success since practitioners can design suitable health promotion programmes by understanding the components that relate to the targeted participants, such as socio-economy, culture, environment, and many more (Poland, Krupa & Mccall, 2009).

The majority of studies found used anthropometric measurements to assess participants' body composition, such as BMI, waist circumference, waist-to-height ratio, waist-to-hip ratio, and body fat percentage. Anthropometry is widely used to assess adult's nutritional status, particularly in large populations (Gómez-Campos *et al.,* 2021), making it suitable for detecting obesity (Low *et al.,* 2020; Motamed *et al.,* 2017). It is a simple yet reliable measurement for predicting cardiovascular disease risk factors (Liu *et al.*, 2019; Zeng *et al.*, 2014), cardiometabolic diseases, and hypertension (Zhang *et al.*, 2013).

The study duration identified was between less than 3 months and up to 24 months, depending on the objectives. However, since cross-sectional research design is limited to one-time data collection, so although it can investigate cause-and-effect relationships between independent and dependent variables (Rogers & Revesz, 2019), it is unable to demonstrate further explanation for temporal relationships and causality in the long run (Zeng *et al.*, 2014). On the other hand, using experimental design gives researchers an advantage in testing variables between control and experimental groups (Rogers & Revesz, 2019), despite the fact that it takes longer time for data collection. Therefore, by mapping the characteristics and health measurements, we hope to assist researchers in choosing suitable variables for future health promotion programmes.

Theory plays a vital role in understanding the complexities of humans. As a consequence, a variety of theories and models are established, targeting different components in health promotion programmes (Raingruber, 2017). It is crucial to look into a broader perspective that influences human health, like psychology, culture, organisation, community, politics, and policy (Raingruber, 2017). However, one theory or model cannot possibly cover every perspective in health promotion.

Only HBM, HPM, and HSET were initially considered for health-related behaviours from the 13 theories and models listed. In comparison, the rest were adapted accordingly to fit the health-related context. Several theories can be categorised into behavioural change theories such as SCT, TPB, SDT, TTM, HBM, and TRA (Noar & Mehrotra, 2011; Raingruber, 2017). Despite criticism for excluding socio-cultural factors, the economy, policy, and the environment (Raingruber, 2017), these theories and models are still widely used in health behavioural research.

Some studies were focused on changing the behaviour of the participants, such as intention (TPB, RAA, and HOEM), self-reflection and self-organisation (SCT), intrinsic motivation (SDT), self-regulating behaviours, and self-initiated reconfiguration (HPM).

Meanwhile, similar constructs like self-efficacy can be found in SCT, TTM, HBM, SET, HSET, and HOEM. Self-efficacy is a person's belief that he/she can change his/her behaviour. Thus, it is crucial to initiate any behavioural changes in order to successfully create a positive outcome (Ajzen, 1985; Bandura, 1999; Prochaska & Velicer, 1997). The positive outcomes from the extracted articles were proven, like improvement in health behaviours or having great potential for future studies.

Some theories, such as TTM, have been useful in determining a person's readiness to change his/her health behaviour by stages or phases. For example, a study by Ott *et al.* (2015) and Abdi *et al.* (2015a) showed a positive change among participants in the pre-contemplation, contemplation, and preparation stages. A study by Romain *et al.* (2018) indicated that interaction between stages could predict physical activity levels among participants. Meanwhile, some studies showed an improvement in weight reduction (Armitage *et al.*, 2017; de Menezes *et al.*, 2015) and had great potential to help overweight or obese adults lose weight by using digital health interventions (Silberman *et al.*, 2020) or text message intervention (Lin *et al.*, 2015). Hence, TTM is useful to predict or deliver a positive outcome in a health promotion programme.

Limited studies took into consideration social or community influences, yet they gave significant outcomes. For example, previous studies guided by SCT found that social influence from family and friends improved physical performance activity and led to weight reduction (Abdi *et al.*, 2015b; Griffin *et al.*, 2018; Hales *et al.,* 2016). A similar result was found in Schifferdecker *et al.* (2016), which showed that encouragement and social support from family and neighbours resulted in increased physical activity and exercise. Meanwhile, a study by Powers *et al.* (2019) indicated a supportive community was able to bring positive changes in participants' healthy choices and eating behaviours.

This scoping review comes with some strengths and limitations. Past research showed the significance of using theories or models in public health interventions like health promotion programmes. Hence, this article's intent was to gather information that will help researchers and practitioners choose a suitable theory or model to be implemented in their future research. Although using theories or models is a familiar practice in health promotion programmes, there are limited review papers that gather these two variables. While scoping reviews are not mainly used to appraise the quality of the extracted studies, we hope this article might provide a head start to researchers who want to further examine the quality of using theories and models in health promotion programmes.

Apart from not accessing the quality of gathered articles, this scoping review was only limited to four databases from the years 2015 until 2021 with specific inclusion and exclusion criteria. Hence, we advise future researchers to do more comprehensive searching by increasing the number of databases and years of study. In addition, conducting scoping reviews requires more reviewers in order to accelerate the process. Nevertheless, a minimal number of reviewers is able to minimise discrepancies in data selection and charting.

CONCLUSION

This review provided an insight that applying theories and models can contribute to the effectiveness of health promotion programmes, particularly for the prevention of overweight and obesity. In addition, future health promotion

programmes need to understand the characteristics of the participants in order to incorporate suitable constructs and components into the programme. With these considerations, it will increase the chances of a successful programme in combating the prevalence of overweight and obesity among adults.

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Authors' contributions

TS, designed the study, screening, analysed, and prepared the manuscript; SWWSSTW, structured, edited and reviewed the manuscript; HFMR, structured, edited and reviewed the manuscript; RAT, assisted in screening, methodology, structured, edited, and reviewed the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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Comparison of boba pearls made from tapioca starch and other unconventional flours and starches: Their glycaemic response (GR)

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ABSTRACT

Introduction: Boba milk tea, also recognised as bubble tea, is a popular beverage in Asia. The primary component in bubble tea is "boba" or "pearl" balls, made of tapioca starch. However, much remains to be seen if tapioca boba pearls have a profound impact on blood glucose. **Methods:** In a randomised, controlled cross-over, single-blinded design study, 12 healthy Chinese male adults (body mass index 21±14 kgm^{-2}) attended four sessions. At each session, bubble tea consisting of boba pearls made from tapioca starch (TS), sago starch (SS), high-amylose starch + sago starch (HA), or kithul flour + sago starch (KF) were served. Boba milk tea was served at breakfast, with volunteers consuming them in a fasted state at each session. The postprandial glycaemic response and insulin response were compared within participants. **Results:** There were observed differences at time 180min for incremental glucose between HA and SS (p=0.005), and for TS and SS for incremental insulin (p=0.004). Glucose iAUC was lower for TS compared to the other boba pearl treatments, although not significantly (p=0.093). There was no significant difference in iAUC of insulin (p=0.104) between the four boba pearl milk teas. **Conclusion:** With limited scientific research conducted on bubble milk tea, our study was the first to document the glycaemic responses of tapioca starch boba pearls and boba pearls made using unconventional flours and starches. The findings from this study is an important first step for future work to develop healthier boba pearls for bubble tea.

Keywords: boba pearls, flours, glycaemic response, insulinaemic response, starches

INTRODUCTION

Boba milk tea, otherwise recognised as bubble tea, has gained immense popularity globally, especially in Asia. The primary component in this beverage is "boba pearl". Boba pearl is commonly made from tapioca starch and has a chewy texture after cooking. It is usually soaked in a sugar syrup, after which is added to a sucrose-sweetened milk tea just before being served. The health implications of consuming boba milk tea, on its own, has been reported in a few studies, with a focus on the implications

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for obesity risk due the sugars present in the beverage (Min, Green & Kim, 2017; Pei *et al.*, 2018). Hence, boba milk tea is sold with varied proportions of sugar, so that consumers are given an option to reduce sugar level or to have it with no sugar.

Tapioca boba pearls not only increase the calories in bubble tea, but tapioca starch (TS) is putatively high in glycaemic index (GI) (Ramdath *et al.*, 2004; Remya, Jyothi & Sreekumar, 2018). It is known that high GI foods elicit a relatively large postprandial rise in glucose and insulin levels (Brand-Miller *et al.*, 2009; Ludwig, 2002). With the consumer shift towards healthier foods and a concomitant increase in the consumption of boba milk tea, it is crucial to produce alternatives for tapioca pearls. This will enable manufacturers to improve the overall nutritional properties of boba milk tea.

Tapioca pearl (made from tapioca starch) is derived from the roots of a cassava plant (*Manihot esculenta*) (Tonukari, 2004). Starch is an essential food ingredient in many food products, especially in Asia, where many food products are highly carbohydrate-based. For example, sago starch (SS) is used in the production of biscuits, noodles and bread (Karim *et al.*, 2008), and more than two-thirds of cassava (tapioca) starch production is used for human consumption (Tonukari, 2004).

Therefore, there is a need to produce starchy foods that are low in GI, i.e. slowly digested, thereby leading to a small and gradual rise in blood glucose. In a previous study, Ng & Henry (2020) studied the physiochemical characteristics of unconventional starches used in Asia and it was found that high amylose maize was highly stable, requiring higher gelatinisation temperature, while kithul flour (KF) (*Caryota urens*) had a digestion rate that was significantly lower than other conventional starches/flours.

The postprandial glycaemic responses (GR) of consuming bubble tea have not been tested; this study therefore will pave the way to formulate 'healthier' and palatable forms of boba pearls for consumers. In selecting our alternative sources of carbohydrate, it was important to recognise and be cognisant of the unique texture and mouthfeel of the tapioca-based boba pearls. Therefore, the alternative carbohydrate sources investigated were: KF (*Caryota urens*), SS (*Metroxylon sagu*), and high amylose maize starch (HA). These carbohydrates were chosen on the basis of their wide availability and application in producing a variety of Asian-based snack foods.

Taking into consideration the above factors, this study, for the first time, aims to compare the GR of conventional tapioca boba pearls and other boba pearls formulated using unconventional flours and starches. These different boba pearls will be formulated in a standardised milk tea concoction and consumed with a snack, as a holistic eating event.

MATERIALS AND METHODS

Boba pearl flours and starches

TS (Ng Nam Bee Marketing, Singapore), SS (Yiak Say Hang Food Industries, Singapore), HA (HI-MAIZE® 260, Ingredion, Singapore), and KF (Kandy, Sri Lanka) were used in the making of boba pearls.

Boba pearl making

Preliminary work was done using a systematic method for the formulation of boba pearls. Boba pearls were made by mixing the starch or flour with boiling water according to the formulation stated in Table 1. The mixture was kneaded to form a dough which is rolled and shaped into a long and thin log (diameter: 1cm). A dough cutter was used to cut the log into small pieces (length: 5mm) and each

piece was rounded into a small ball with an average weight of 0.75±0.05g.

TS and SS were used without any blending as they were able to form a dough-like structure when mixed with boiling water. For the blended variants, a proportion of SS was mixed with KF or HA starch until a perfect dough-like structure was formed when boiling water was added. Cooking time was also pre-determined from these trials to ensure that the starches were cooked thoroughly to an optimal level of chewiness. The amount of cooking time, flour(s) and water used for each treatment were recorded and shown in Table 1.

Total starch and amylose content

The total starch content of boba pearls was measured by an enzymatic technique using the Megazyme assay kit (K-TSTA, Megazyme International, Ireland). Samples were first incubated with thermostable α-amylase at 100°C to hydrolyse starch into maltodextrins. For the high amylose maize variant, cold 1.7M sodium hydroxide was used to pre-dissolve the resistant starch present, and sodium acetate buffer was used to neutralise the sample before the addition of thermostable α-amylase. Amyloglucosidase was then added to hydrolyse maltodextrins into D-glucose. For quantitative measurement, glucose oxidase/peroxidase (GOPOD) reagent was then added and the absorbance of the samples was determined using a UV spectrophotometer (UV-2600, Shimadzu, Japan).

The amylose content of the boba pearls was measured using the amylose/amylopectin assay kit (K-AMYL, Megazyme International, Ireland). Samples were first dispersed in dimethyl sulphoxide (DMSO) at 100°C and then ethanol was added to remove the lipids. The precipitated sample was dispersed in DMSO before dissolution in an acetate and salt buffer. An aliquot of

Table 1. Formulation of boba pearls and milk tea

Boba Pearls	*Starch/ Flour 1*	*Amount (g)*	*Starch/ Flour 2*	*Amount (g)*	*Water (g)*	*Cooking time (mins)*	*Cooked weight of pearls (g)*	*Total available CHO (g)*
A: Tapioca Starch (TS)	TS	54.0	-	-	32.4	7	86.4	50
B: Sago Starch (SS)	SS	57.4	-	-	33.3	7	90.8	50
C: Kithul flour (KF)†	KF	41.5	SS	17.8	33.6	7	92.9	50
D: High amylose maize starch (HA)†	HA	38.1	SS	38.1	57.2	13	133.4	50

Milk Tea & Bread	*Amount (g)*	*Total amount (ml)*	*Total available CHO (g)*
Tea	246.8	300	0
Evaporated milk	37.0		4.6
Sugar syrup	16.0		10.7
White bread	47.8	47.8	25

†Blends with sago starch (SS)

the dissolved sample was taken for the measurement of total starch later. To another aliquot of the dissolved sample, lectin concanavalin A (Con A) was added to complex the amylopectin which was removed by centrifugation. An aliquot of the supernatant was taken for the measurement of amylose. An enzyme mixture of amyloglucosidase and α-amylase was added to both the amylose and total starch aliquots for hydrolysis into *D*-glucose. GOPOD reagent was then added and the absorbance of the samples was measured. The concentration of amylose was estimated as the ratio of absorbance of the supernatant aliquot to that of the total starch aliquot.

Total available carbohydrate (TAC) content

The total available carbohydrate (TAC) content of boba pearls was measured by an enzymatic technique using the Megazyme assay kit (K-ACHDF, Megazyme International, Ireland). The flours were first incubated at 80°C with α-amylase to gelatinise, hydrolyse, and depolymerise non-resistant starch. They were further incubated at 60°C with protease to solubilise and depolymerise proteins. Amyloglucosidase was also added to hydrolyse starch fragments into *D*-glucose. Following enzymatic hydrolysis, the absorbance of the samples was measured using a UV spectrophotometer (UV-2600, Shimadzu, Japan) to determine *D*-glucose and *D*-fructose. The TAC (%) present in the flour is derived from the sum of *D*-glucose content (%) and *D*-fructose content (%).

Texture analysis of boba pearls

The texture analysis of cooked boba pearls was analysed by performing two successive compressions using a texture analyser, TA-XTplus (Stable Micro Systems Ltd, Surrey, UK). A single boba pearl sample was compressed to a strain of 75% using a cylindrical probe (Ø = 75 mm) with a trigger force of 5.0 g. The pre-test speed was 1 mm/second (sec) and both the test and post-test speeds were 5 mm/sec. The recovery time was 5 sec between the first and second compression. The analysis was carried out at room temperature. There were ten replicates for each sample. The subsequent parameters were achieved from the force-distance curves (Bourne, 1978): the peak force (N) of the first compression was determined as "hardness"; the ratio of the area of the second and first compression was determined as "cohesiveness"; the ratio of the pearl's detected height in the second compression cycle to that of the first compression was determined as "springiness"; and the multiplication of the hardness, cohesiveness, and springiness values was identified as the "chewiness".

***In vivo* study**

The research took place at the Clinical Nutrition Research Centre (CNRC) within the Singapore Institute for Food and Biotechnology Innovation (SIFBI), Agency of Science, Technology and Research (A*STAR), Singapore. The study was approved by the Domain Specific Review Board of the National Healthcare Group (2018/01194), registered under the Clinicaltrial.gov registry as NCT04115657. All procedures were conducted based on the guidelines stated in the Declaration of Helsinki.

Study population

Volunteers were recruited from the public. Anthropometric measurements (height, weight, waist-hip circumference, triceps and biceps skinfolds), blood pressure, and finger-prick fasting blood glucose measurements were collected. Females were not included to minimise disparities in the data because of hormonal changes during their menstrual cycle. Young, healthy Asian

Chinese males between the ages of 21 - 40 years, with a body mass index (BMI) between 18.5 to 25 kg/m^2 and normal blood pressure (<140/90 mmHg) were included in the study. Those who were athletes/sportsmen, dieters, smokers, with a fasting blood glucose of >6 mmol/L, glucose-6-phosphate dehydrogenase deficiency (G6PD deficiency), metabolic diseases, such as diabetes, hypertension etc., medical conditions and/or taking medications known to affect glycaemia (glucocorticoids, thyroid hormones, thiazide diuretics), intolerances or allergies to foods, were excluded from the study.

Sample size

Studies of the analysis of GR and GI in humans have been based on ten subjects, as reviewed by the Food and Agriculture Organization/World Health Organization (FAO/WHO, 1998) to take into account the inter-individual variations. A sample size of 12 was therefore considered adequate for the current study to account for inter-individual variabilities.

Study design and experimental protocol

The study was a randomised, controlled, single-blinded cross-over design. Participants attended one screening visit and four test sessions (consisting of a milk tea with different types of boba pearls and a snack, which was white bread). For a controlled condition, the tapioca boba milk tea was used. The other three test boba milk teas were tested with similar formulations, except for the types of boba pearls used. All four test sessions were separated by a three-day washout period to minimise any cross-over effects. Randomisation of the sequence of treatments was determined through an online computer software (Randomizer.org).

Volunteers who were interested in the study and fulfilled all the inclusion criteria underwent a screening session. They were requested to come to the centre in the morning (fasted for at least ten hours). Informed consent was signed before basic anthropometric measurements were measured. Height was taken using a stadiometer (Seca Limited, Birmingham, West Midlands, Middlesex, UK), body weight and composition were obtained using the bioelectrical impedance analysis (BIA) machine (Tanita BC-418, Tokyo, Japan), and the Omron blood pressure monitor (Model Hem-907) was used to measure participants' blood pressure. The HemoCue 201+ Glucose RT analyser (HemoCue Ltd., Dronfield, UK) was used to measure finger-prick fasting blood glucose levels. Three days before the test session, volunteers were requested not to take part in rigorous activities and they were also told to avoid caffeine and alcohol the day before the test.

On test days, volunteers had to arrive between 8:30 am to 9:00 am after a 10- to 12-hour overnight fast. They rested for ten minutes (min) before an indwelling intravenous cannula was inserted into a forearm vein, by a phlebotomy-trained state registered nurse. Throughout the test session, the line was kept patent with 3 mL non-heparinised saline. A 3 mL baseline venous blood sample (0 min) was obtained immediately after the insertion of the catheter. Subsequently, the volunteers were asked to consume the boba pearl drink and white bread within 15 min. After the test meal, 3 mL venous blood samples were collected at 15, 30, 60, 90, 120, 150, 180 min to measure plasma glucose and insulin concentrations. Volunteers were encouraged to remain desk-bound throughout the three-hour study period to minimise physical movement. After 180 min, the catheter was removed and

the study session was completed. The same steps were repeated for all test visits.

Treatment meals

Volunteers were required to consume a standardised dinner the night before the test session. The standardised dinner consisted of a frozen, ready-to-eat meal (Butter chicken with cumin rice, Chef-in-Box, Singapore) and a Milo tetra pack drink (Milo Chocolate Malt, Nestlé, Switzerland). Given that boba tea is a complex mixture of boba pearls, milk tea (that contains sucrose), and is usually consumed with a snack (a slice of bread), we decided to simulate the real-life situation in our experimental design. For each test day, participants consumed boba pearl milk tea along with white bread as a meal. Therefore, the amount of available carbohydrates provided by boba pearls was 50g, milk tea 10.7g, and bread 25g (Table 1).

All boba pearl milk teas were prepared in the CNRC food product development kitchen. Before each test day, boba pearls were prepared and then stored in a 4°C chiller. On the test day, these chilled boba pearls were then boiled in water according to a pre-determined cooking duration. After cooking, the pearls were strained into the serving cup, allowed to cool for a minute in an ice bath, after which chilled milk tea was added and the beverage was served immediately.

For the tea base of the drink, 12g tea leaves (Brooke Bond 3 roses, Unilever, UK) was boiled in 1.1L of water for one min and then simmered for another three min. Then, 150g of evaporated full cream milk (Carnation, F&N Holdings, Malaysia) was added into 1L of brewed tea together with 65g of 200% w/w sugar solution (SIS, Singapore). The milk tea was also made the day before and stored in a 4°C chiller. The formulation for one serving (300 g) of milk tea was 247g of brewed tea, 16g of sugar solution, and 37g of evaporated milk.

Blood analysis

Venous blood samples were collected at fixed time points in Vacutainers® (Belton Dickinson Diagnostics, NJ, USA) with disodium EDTA. These samples were then centrifuged at 1500 g for 10 min at 4°C (Sorvall™ ST 16 Centrifuge, Thermo Fisher Scientific, Waltham, MA, USA), where plasma was obtained. Plasma was aliquoted into Eppendorf tubes and stored at -80°C until analysis. Plasma glucose was measured using the immunochemistry analyser COBAS c311 (Roche, HITACHI, Los Gatos, CA, USA), while plasma insulin was measured using the immunochemistry analyser COBAS e411 (Roche, HITACHI, Los Gatos, CA, USA). Inter- and intra-assay *CV*s for glucose (<2% and <1.5%, respectively) and insulin (<6% and <5%, respectively) were determined by the manufacturers. Postprandial blood glucose concentration changes were measured by computing the difference between the fasting and the blood glucose concentration at a specific time interval. The trapezoidal rule was used to calculate postprandial glucose and insulin incremental area under the curve (iAUC), ignoring the area under the baseline (Wolever, 2006).

Data and statistical analysis

All data and figures were processed in a Microsoft Excel spreadsheet (Microsoft Corporation), presented as mean±*SEM* (standard error of the mean), unless otherwise stated. Data were tested for normality using the Sharpiro-Wilk test, as well as visually using Q-Q plot. Linear mixed effects procedure with treatment as fixed factor and subject as random effect was conducted to investigate the effect of treatment on the iAUC for glucose and insulin. The same procedure was used

Table 2 Total starch, amylose and total available carbohydrate contents of boba pearl samples

Boba pearl sample	*Total starch (% w/w d.w.b)*	*Amylose (% w/w d.w.b)*	*TAC (%)*
TS	86.4±1.6	23.0±2.1	92.6±0.1
SS	85.2±0.5	24.3±0.4	87.0±0.9
KF+SS	88.7±0.6	20.6±2.2	83.1±1.1
HA+SS	90.1±4.5	40.5±2.0	53.3±1.1

TS, tapioca starch; SS, sago starch; KF, kithul flour; HA, high amylose maize starch

to test for significant difference in mean fasting glucose and insulin values prior to the four treatments, as well as at each time point of the incremental glucose and insulin responses. Statistical significance was attained when $p<0.05$. All statistical analysis was done using IBM SPSS for Windows version 24.0 (IBM Corp, Armonk, NY, USA).

RESULTS

Chemical composition of raw materials

The chemical composition of all starches and flours used in the formulation of the boba pearls is derived from our previous work (Ng & Henry, 2020). All the starches contained trace amounts of proteins, except for HA. There was trace amounts of fat for SS and KF boba pearls. A high dietary fibre content was observed in KF and HA boba pearls.

Total starch, amylose content, and TAC content of boba pearls

Total starch, amylose content, and TAC content of the boba pearl samples are shown in Table 2. The amylose content in HA boba pearls was almost twice the amylose content of the other boba pearl variants. The TAC analysis was conducted to determine the available carbohydrate content, so as to calculate the available carbohydrate of treatment meals for *in vivo* GR testing.

Texture analysis of boba pearls

The hardness, cohesiveness, springiness, and chewiness for the four types of cooked boba pearls were determined (Table 3). HA had much higher hardness, lower cohesiveness, and lower springiness as compared to the TS boba pearls. SS had similar textural parameters as TS boba pearls. KF boba pearls had higher hardness and chewiness compared to TS and SS pearls.

In vivo study

Baseline characteristics

For the present study, 12 young, healthy Chinese male adults fulfilled the study inclusion criteria and completed all four arms of the study. Their baseline characteristics are shown in Table 4.

Table 3 Texture parameters measured for boba pearls

Boba pearl sample	*Hardness (N)*	*Cohesiveness*	*Springiness*	*Chewiness*
TS	10.32±1.05	0.86±0.02	0.94±0.05	8.41±1.09
SS	11.62±1.54	0.86±0.01	0.95±0.04	9.44±1.30
KF	16.65±2.57	0.84±0.01	0.96±0.04	13.49±2.36
HA	28.57±2.51	0.47±0.01	0.72±0.05	9.80±1.51

TS, tapioca starch; SS, sago starch; KF, kithul flour; HA, high amylose maize starch

Table 4 Characteristics of study participants (n=12). Data presented as mean±*SD* (standard deviation)

Anthropometric and physiological parameters	*Mean±SD*
Age (years)	24.8±1.8
Height (cm)	173.9±6.4
Weight (kg)	64.7±7.4
BMI (kg/m^2)	21.3±14.8
Systolic blood pressure (mmHg)	122.7±8.0
Diastolic blood pressure (mmHg)	73.2±6.6
Waist circumference (cm)	74.1±5.1
Hip circumference (cm)	94.5±4.8
Fasting blood glucose (mmol/L)	4.5±0.4

BMI, body mass index

Glucose and insulin responses

Postprandial glucose responses of the four treatments are shown in Figure 1A and the postprandial insulin responses to the four treatments are shown in Figure 1B. All treatments produced an early rise in plasma glucose concentration with TS and HA having the highest peak level around 30 min, while SS and KF showed a later peak for blood glucose, i.e. 45 min after consumption followed by a gradual decline (Figure 1). At the 180min time point, there was a significant difference between HA and SS for incremental glucose (p=0.003). When presented as iAUC, TS appeared to have the lowest reduction in glucose response, but overall no significant difference was found between all treatments (p=0.093). There were significant differences observed in incremental insulin at time points 90 (p=0.031) and 180min (p=0.004). At 180min, incremental insulin values of SS was significantly lower than that of TS (p=0.016) and HA (p=0.006). However, after Bonferroni correction, there was no significant difference between the treatments at time point 90min. In addition, the iAUC for insulin (p=0.104) did not show significant differences between the four boba pearl milk teas.

DISCUSSION

There is a growing demand for starchy foods to be more slowly digested and metabolised, leading to a more gradual and smaller rise in blood glucose. Hence, the purpose of this study was to investigate whether tapioca starch boba pearls may elicit a high GR and whether boba pearls developed from unconventional starches may reduce the GR compared to tapioca boba pearls. Given boba tea's wide popularity, and the potential impact of consuming high GR foods on metabolic health, our study was a first attempt to examine the postprandial glycaemia of boba milk tea.

Before commencing *in vivo* work, alternative starches and flours for new boba pearls were formulated for comparison with tapioca boba pearls. Our previous work showed KF and HA starches to have significantly lower rates of digestibility as compared to the other starches, explained by the higher amounts of protein, amylose, and dietary fibre present in them (Grace *et al.*, 2020). Hence, these flours and starches were selected to create new boba pearls. The formulation of boba pearls was conducted in a systematic way to ensure that a perfect dough-like

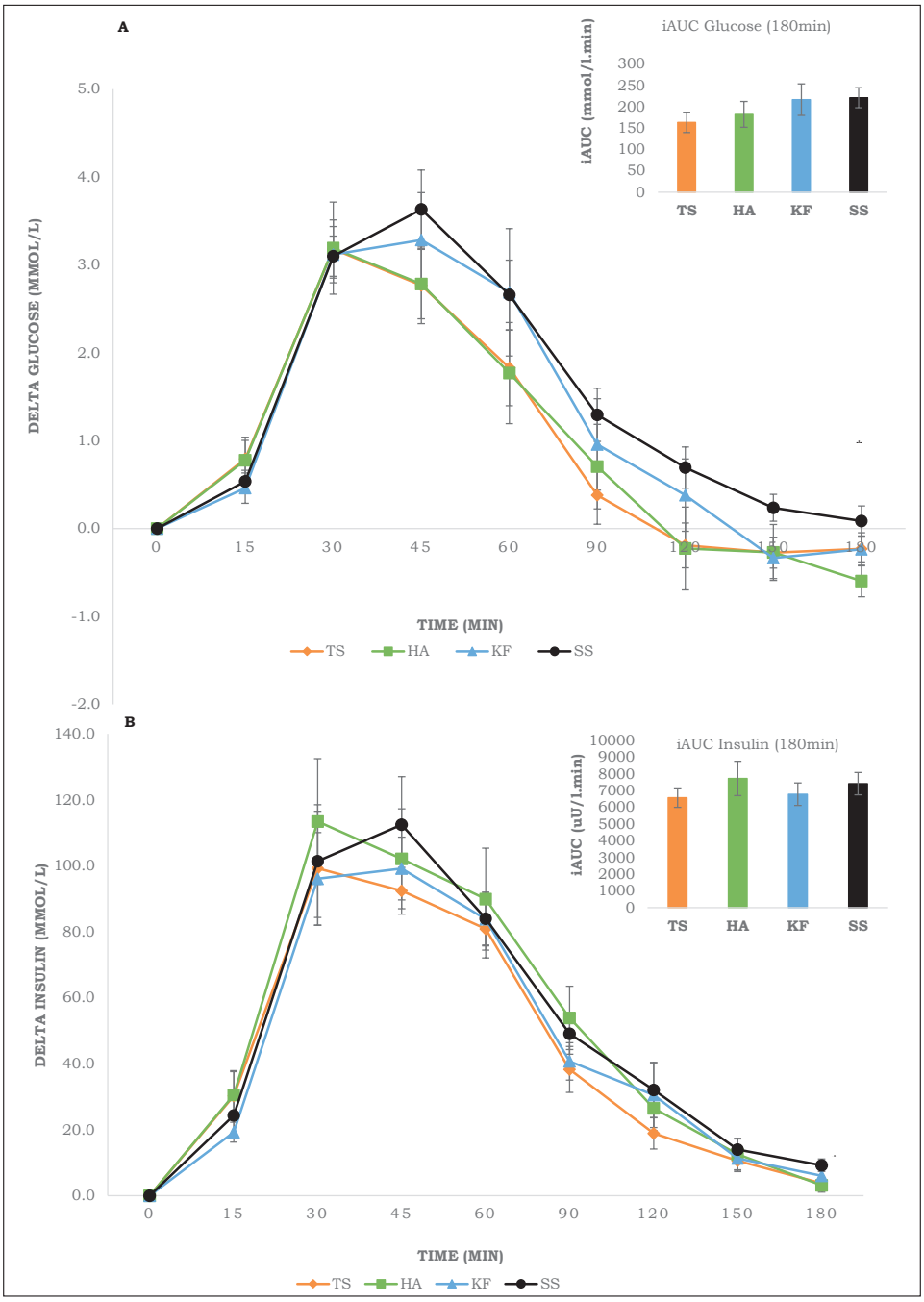

Figure 1
represents the incremental glucose (A) and insulin (B) curves for 180 min. The bar plots on the right-hand side represent values as mean±SEM; *n*=12. iAUC180 was calculated using the trapezoid rule ignoring the area below the baseline. Total iAUC180 corresponds to the area under the curve for the entire 180 minutes of measurement. $p>0.05$* indicates time point at which there was significant difference between the incremental values.
TS, tapioca starch; HA, high amylose maize starch; KF, kithul flour; SS, sago starch

structure was formed. This was a crucial step in ensuring that the boba pearls formed using alternative sources of carbohydrate were cognisant with the textural qualities of tapioca starch boba pearls. TS and SS were able to form without any blending. On the other hand, HA and KF were blended with SS. The lower starch content, and the higher fat, protein, and dietary fibre contents in these flours, as well as the higher amylose to amylopectin ratio in HA resulted in higher pasting temperatures and decreased gelatinisation (Grace *et al.*, 2020, Tian *et al.*, 2019). This contributed to the inability for HA and KF starches to form a dough-like structure when mixed with boiling water. Therefore, to form boba pearls using KF and HA, SS was required in the blend to act as a binder in order for these variants to be able to form a dough-like boba pearl perfectly.

Textural parameter findings showed that HA boba pearls had much higher hardness, lower cohesiveness, and lower springiness as compared to TS boba pearls. This may be attributed to the incomplete gelatinisation and restricted swelling of the starch granules due to limited water absorption in the HA boba pearls (Cornejo-Ramírez *et al.*, 2018). Compared to TS and SS, KF had a greater amount of protein and dietary fibre, which contributed to its higher hardness. Although there were some differences in the texture of the boba pearls, the instrumental measurement could be more sensitive in distinguishing textural properties than the sensory perception in humans (Truong *et al.*, 2002).

The iAUC glycaemic response results although not statistically significant, saw an overall greater attenuation of glucose response with TS and SS compared to TS and HA. Time point 180 min saw significant differences in glucose response between HA and SS (p=0.003). This could be due to the large amount of dietary fibre (resistant starch) and high amylose content present in HA that may have a large influence on attenuating the glucose response (Ingredion, 2020). SS and KF have lower amylose content, with SS containing between 24 to 31% amylose content (Ahmad *et al.*, 1999). This could be a possible explanation for the higher glucose iAUC observed in SS and KF compared to HA and TS boba pearls. This indicates that the amylose content of starches and flours used in formulating boba pearls may play an important role in attenuating glucose response. The molecular structure of amylose is tighter and more compact, thus less susceptible to breakdown than amylopectin whose structure is more vulnerable to digestion. Therefore, the amylose content of starches and flours may influence both the textural qualities and glucose attenuating properties of boba pearls. This may be an important consideration when developing and formulating boba pearls.

A possible explanation for the treatments showing no statistical significance could also be due to the combination of consuming other non-starch ingredients together that may have an interaction with boba pearls. Non-starch ingredients, such as tea, which is rich in polyphenols, such as catechins and tannins, may decrease starch digestibility and possibly blood glucose response by inhibiting enzymes and interacting with starch (Thompson & Yoon, 1984). Future studies should consider using a liquid-base component that is not rich in polyphenols, and/or other ingredients known to affect glycaemia.

Differences in the habitual mastication of starchy foods can also contribute to glycaemic variations (Ranawana *et al.*, 2011, Ranawana *et al.*, 2010). Volunteers were asked to

consume boba milk tea with a large straw, through which the boba pearls were slurped and chewed. However, the number of chews per boba pearl was not controlled for during this study. Hence, this could be a confounder in the study as factoring the number of chews each volunteer takes to masticate each pearl is different and this could have affected the rate of starch digestion. It is also unclear if the small textural differences between the boba pearls may have an effect on the masticatory sequence, which may have influenced the GR of the chewed particles (Bornhorst & Singh, 2012, Miwa, Shiga & Kobayashi, 2001). Thus, in addition to replacing the liquid-base component of the beverage, future studies could also focus on the mastication process of boba pearls in determining the glycaemic response of a boba drink. Finally, given that there is some contribution of lactose from evaporated milk in bubble tea, a criteria to test participants for lactose intolerance should be included. A small number of Asian Chinese might have the ability to break down lactose and this may contribute to postprandial glucose response. These factors and limitations may have led to differences in glycaemic and insulinaemic responses, and should therefore be considered for future work in this area.

CONCLUSION

Building on a real-life situation where boba tea is a complex mixture of pearls and milk tea, consumed with a snack, our study was the first of its kind to examine the effects of various boba pearls when consumed as a holistic eating event. Using tapioca starch and a range of unconventional flours and starches in boba pearl manufacture, our study showed that there was no statistical significance, but the results were nonetheless still meaningful and warrants further investigation. An understanding of the impact on GI would make it more useful to gauge its practical relevance. Our findings enhanced the research in this field and is a necessary first step in understanding how this popular beverage fits in the landscape of sugar-sweetened beverages in the Asian region. With limited published data on the GR of boba pearls consumed in this region, this study is timely as it sets a firm foundation upon which future work can be based on.

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Authors' contributions

BK, implemented and performed the experiments and human studies, analysed and interpreted the data, responsible for the statistical analysis and interpretation of data, and wrote the manuscript; RYCQ, implemented and performed the experiments and human studies, analysed and interpreted the data, responsible for the statistical analysis and interpretation of data, and wrote the manuscript; GCFN, responsible for the conception and design of the study, implemented and performed the experiments and human studies, analysed and interpreted the data, and wrote the manuscript; SP, analysed and interpreted the data, responsible for the statistical analysis and interpretation of data, and wrote the manuscript; CJH, responsible for the conception and design of the study, and wrote the manuscript. All authors read and approved the manuscript and had full access to the study data and shared the final responsibility for the decision to submit this report for publication.

Conflicts of interest

All authors declare no conflict of interest.

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Whey protein positively alters inflammatory markers and metabolic parameters of overweight and obese adults

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ABSTRACT

Introduction: The effects of prolonged consumption of whey protein on health are controversial. This study aimed to determine whether whey protein positively alters health parameters of overweight and obese adults. **Methods:** Randomised controlled trial was conducted. Fifty-eight participants, aged 30-50 years, were randomly allocated into four groups and supplemented with 50 g protein for eight weeks (group 1: plant-based protein (PBP), group 2: whey protein isolate (WPI) with cocoa powder, group 3: PBP with whey protein concentrate (WPC), and group 4: WPI with milk powder). Body composition and biochemical parameters (kidney and liver functions, inflammation, oxidative stress, and antioxidant capacity) were evaluated at pre-intervention and 8 weeks after intervention. **Results:** At Week 8, group 3 had lower diastolic blood pressure, waist circumference, visceral fat, and risk of insulin resistance ($p<0.05$ for all). Group 2 had decreased levels of total cholesterol and low-density lipoprotein cholesterol ($p<0.05$ for all). A drop in triglyceride was seen in group 4 ($p=0.026$). Whey protein decreased alanine aminotransferase level ($p=0.028$), while PBP increased aspartate aminotransferase level ($p=0.034$). PBP or WPI with milk powder increased blood urea nitrogen level ($p>0.05$ for all). Interleukin-6 and lactoferrin levels fell in all groups ($p<0.05$), while hs-CRP increased in the PBP group ($p=0.043$). Group 2 experienced increased antioxidant capacity. However, levels of oxidative stress markers were significantly decreased in the PBP group and WPI with milk powder group. **Conclusion:** Whey protein revealed positive effects on anthropometric parameters and biochemical markers of overweight and obese adults. Therefore, proper supplementation of whey protein can potentially promote health.

Keywords: inflammatory marker, obesity, overweight, plant-based protein, whey protein

INTRODUCTION

Overweight and obesity is a substantial public health problem, and its global prevalence has continually increased (World Obesity Federation, 2021). The latest Thai national survey reported that the prevalence of obesity among those aged over 15 years was 42.0%

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in females and 33.0% in males, and these figures will likely increase every year (Aekplakorn & Thai National Health Examination Survey Office, 2016). Obesity is reportedly associated with inflammation. Accumulation of fat cells in obesity can stimulate the secretion of acute phase reactants and pro-inflammatory cytokines, such as C-reactive protein (CRP), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α), consequently triggering oxidative stress and increasing the risk of non-communicable diseases (de Heredia, Gómez-Martínez & Marcos, 2012; Brimelow *et al.*, 2017).

Proper dietary intake plays a crucial role in the prevention and reduction of the severity of obesity and its related diseases. Presently, the role of functional food in the prevention and mitigation of chronic diseases has been widely studied (Pal & Radavelli-Bagatini, 2013). Whey protein is rich in branched-chain amino acids, which are essential for building muscle, reducing muscle injury and muscle fatigue (Witard *et al.*, 2014; Jackman *et al.*, 2017; Shimomura *et al.*, 2010). It has also been found to improve antioxidant capacity and reduce oxidative stress in the body (Zhenyukh *et al.*, 2017). A previous study revealed that consumption of 0.5 g whey protein/kg body weight/day for 16 weeks, versus no whey protein, decreased the body weight and fat mass of obese individuals who had gastric surgery. However, levels of blood glucose and inflammatory indicators, such as IL-6 and adiponectin, did not change (Gomes *et al.*, 2017). Likewise, fat mass and uric acid concentration in diabetes and pre-diabetes patients were remarkably reduced after intake of whey protein, while blood pressure, inflammatory markers, antioxidant capacity, and oxidative stress were not altered (Flaim *et al.*, 2017). Additionally, a recent study has found that consumption of whey protein is associated with the secretion of satiety hormones (Chungchunlam *et al.*, 2015).

However, based on the outcomes of previous studies, the efficacy of whey protein on the health of individuals who are at risk of obesity and non-communicable diseases is not entirely clear. Therefore, this study aimed to evaluate the effect of whey protein on the clinical health of adults. We specifically investigated whether consumption of whey protein resulted in alterations of body composition and biochemical parameters, including blood sugar, lipid profiles, liver and kidney functions, inflammation, antioxidant capacity, and oxidative stress.

MATERIALS AND METHODS

Study subjects

Sixty overweight and obese Thai adults [body mass index (BMI) 23-30 kg/m^2, classified by the Steering Committee of the Regional Office for the Western Pacific Region of WHO, 2000], aged 30-50 years old, were enrolled into this single-blind randomised controlled trial. Exclusion criteria included having chronic diseases, any infection or inflammation six months prior to the study, currently taking medication or nutritional supplements, smoking, regularly drinking alcohol, pregnant or lactating. The subjects were asked to complete an online screening questionnaire and present their annual medical check-up report before participating in the study. Study participants were informed of the risks, discomforts, and benefits associated with the study before providing their signed informed consent. The study procedure was approved by the Ethics Committee of the Faculty of Public Health, Mahidol University, Thailand (Certificate of Approval No. MUPH 2020-215) and was registered with the Thai Clinical Trials Registry (Registration

number TCTR20210721004). Stratified and block randomisation was utilised to allocate the study subjects into four groups.

Supplement characteristics and study intervention

Each study group received different supplements contained in an aluminium foil sachet. Group 1 received plant-based protein (PBP), group 2 received whey protein isolate (WPI) with cocoa powder, group 3 received PBP with whey protein concentrate (WPC), and group 4 received WPI with milk powder. The PBP supplement mainly contained 80.6% isolated soy protein, 10.0% isolated wheat protein, and 7.5% isolated pea protein; the WPI with cocoa powder supplement consisted of 84.9% WPI, 8.5% cocoa powder, and 4.8% mixed amino acids. The PBP with WPC supplement mainly contained 38.9% isolated soy protein, 16.7% fish collagen peptide, 5.6% WPC, and 5.6% malt extract powder. The main composition of the WPI with milk powder supplement was 82.4% WPI, 8.2% milk powder, and 2.7% premixed vitamins and amino acids. The total protein content of the supplements given to the participants was 50 g/day. All participants were asked to continually consume the received supplement for eight weeks.

Study parameters assessment

Dietary intake was recorded three times a week (two weekdays and one weekend) using a food record. To monitor the intakes of study supplements and diet, subjects were asked to take photographs of their food items before and after intake. Additionally, trained staffs randomly called the subjects once a week to inquire about their dietary intake. Energy and macronutrient intakes were estimated by the NutriSurvey programme (Copyright© 2007, SEAMEO TROPMED RCCN-University of Indonesia, Indonesia). Participants underwent anthropometric assessment and biochemical evaluation at pre-intervention, and after the 8-week intervention. Body weight, body mass index (BMI), body fat mass, percentage visceral fat, and muscle mass were assessed by a body composition analyser (DC-360, Tanita Corporation, Japan). Waist circumference (WC) was measured at the umbilical level.

Participants were requested to fast at least 12 hours before blood sampling. A Cobas® 6000 analyser (Roche Diagnostics Ltd., Switzerland) was utilised to evaluate levels of fasting blood glucose (FBG), lipid profiles (total cholesterol, high-density lipoprotein cholesterol: HDL-C, low-density lipoprotein cholesterol: LDL-C, triglyceride: TG), and kidney and liver function markers (aspartate aminotransferase: AST, alanine aminotransferase: ALT, blood urea nitrogen: BUN, creatinine, uric acid). Fasting insulin was examined using a human insulin ELISA kit (ab200011, Abcam, Cambridge, UK). To determine insulin resistance, the homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as follows: HOMA-IR = [Fasting insulin (μIU/mL) × FBG (mmol/L)] / 22.5. High-sensitivity CRP (hs-CRP) concentration was determined by the nephelometry method. Concentrations of lactoferrin, IL-6, and TNF-α were measured using the enzyme-linked immunosorbent assay technique. Antioxidant capacity was evaluated by using an oxygen radical absorbance capacity (ORAC) assay kit (ab233473, Abcam, Cambridge, UK). To determine oxidative stress, a lipid peroxidation assay kit (ab118970, Abcam, Cambridge, UK) was used.

Statistical analysis

Sample size was calculated using G*power programme. To detect the

difference of -1.4±0.9 kg in body fat and -1.7±1.5 cm in waist circumference, with 80% power and α=0.05, the minimum number of participants in each study group was 10. The calculated sample size was increased by 20% to prevent missing data, subject withdrawals, etc. Statistical analysis was performed using Statistical Package for Social Science version 18 (SPSS, SPSS Inc., Chicago, USA). One-way analysis of variance (ANOVA) and Tukey's post-hoc test was conducted to determine differences among the four study groups. The differences between pre- and post-intervention within each study group were evaluated using paired-sample *t*-test. Data were presented as mean ± standard deviation (SD). A $p<0.05$ was considered to be statistically significant.

RESULTS

Baseline characteristics of study subjects

Seventy overweight and obese adults were screened according to the inclusion and exclusion criteria, of which 60 participants were recruited into the study and allocated into four study groups. During the study, two participants declined to participate due to personal reasons. Thus, a total of 58 screened participants completed the 8-week intervention (97% retention rate) (Figure 1). The remaining participants at the end of the study were 93% for group 1 and group 3, and 100% for group 2 and group 4. Group 1 consisted of 14 participants (7 males and 7 females), group 2 consisted of 15 participants

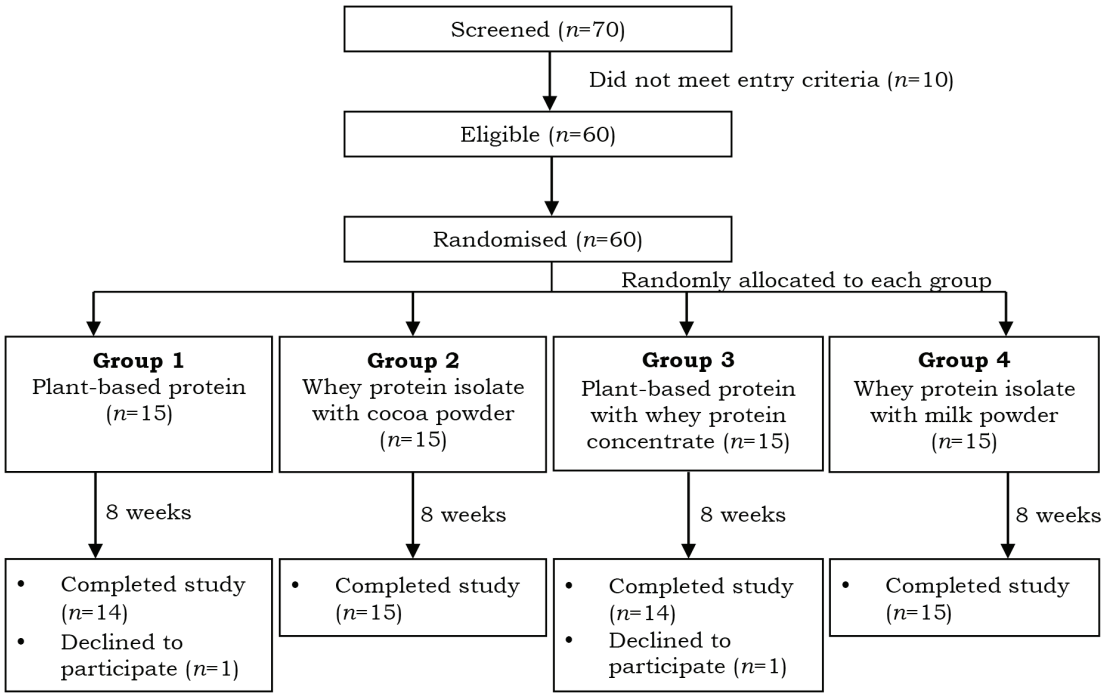

Figure 1. Flow diagram of the study

(3 males and 12 females), group 3 consisted of 14 participants (1 male and 13 females), and group 4 consisted of 15 participants (3 males and 12 females). Mean age of the participants was 43.1±4.1 years (group 1: 44.5±3.6 years; group 2: 43.4±5.0 years; group 3: 41.1±3.8 years; group 4: 42.6±3.6 years, p=0.239).

Effects of whey protein consumption on blood pressure and anthropometry

One hundred percent of the participants in each group consumed the supplement according to the study requirements. All participants underwent blood pressure and anthropometric assessment. Changes in blood pressure and anthropometric parameters are presented in Table 1. Blood pressure of the four study groups at pre-intervention and after the intervention was comparable (p>0.05 for all). Group 3 had significantly lower diastolic blood pressure at Week 8 (*MD*: -3.5±5.8 mmHg, p=0.049). WC of group 3 and group 4 was remarkably reduced after intake of the supplement for 8 weeks (*MD*: -3.3±3.9 cm, p=0.010 and -2.6±3.7 cm, p=0.017, respectively). A significant difference between the study groups existed regarding percentage visceral fat (p=0.024). Comparing within study groups, percentage visceral fat was significantly lower in group 3 after completion of the study (*MD*: -0.3±0.4%, p=0.019). All study groups showed a tendency towards lower fat mass and higher muscle mass after the intervention.

Effects of whey protein on biochemical parameters

FBG concentrations of group 1, group 2, and group 4 significantly increased after the study (*MD*: 0.2±0.4 mmol/L, p=0.048; 0.1±0.1 mmol/L, p=0.002; 0.2±0.3 mmol/L, p=0.035, respectively), while fasting insulin of group 2, group 3, and group 4 significantly decreased (*MD*: -28.0±41.0 pmol/L, p=0.024; -26.3±34.0 pmol/L, p=0.016; -15.2±24.4 pmol/L, p=0.036, respectively), as shown in Table 2. In addition, the HOMA-IR of group 3 was remarkably decreased at Week 8 (*MD*: -1.2±1.4, p=0.031). Regarding lipid profiles, group 2 presented a lower level of total cholesterol (*MD*: -0.4±0.5 mmol/L, p=0.007) and LDL-C (*MD*: -0.4±0.5 mmol/L, p=0.004) after the intervention. Likewise, after the study, group 4 showed a significantly lower level of TG (*MD*: -0.1±0.3 mmol/L, p=0.026). Regarding liver and kidney functions, comparing Week 8 with Week 0, AST of group 1 significantly dropped (*MD*: -0.0±0.0 μkat/L, p=0.034), similarly for ALT of group 3 (*MD*: -0.1±0.1 μkat/L, p=0.028). On the contrary, BUN of group 1 and group 4 significantly increased (*MD*: 0.8±1.2 mmol/L, p=0.040 and 0.4±0.8 mmol/L, p=0.042, respectively). A between-group difference in the level of uric acid was observed, with the highest level recorded in group 1 (p=0.008).

Effects of whey protein on markers of inflammation, antioxidants, and oxidative stress

Regarding inflammatory markers, there were significant changes in levels of hs-CRP, IL-6, and lactoferrin, as shown in Table 3. A remarkable increase in hs-CRP level was observed in group 1 after completion of the study (*MD*: 6.5±9.9 nmol/L, p=0.043), while the level of IL-6 of all study groups was reduced (p<0.05 for all). Likewise, lactoferrin significantly decreased in group 1, group 2, and group 3, and tended to decrease in group 4 (*MD*: -259.7±283.3 μg/L, p=0.004; -406.5±332.4 μg/L, p<0.001; -744.9±586.6 μg/L, p=0.001; -317.6±557.9 μg/L, p=0.063, respectively). A noteworthy increase in antioxidant capacity was found in group 2 (*MD*: 1.7±2.6 μM TE/ml, p=0.026), with other groups showing slight increments. Group 1 and group 4 presented a

Table 1. Comparison of blood pressure and anthropometric parameters between Week 0 and Week 8

Variables	*Group 1 (n=14)*	*Group 2 (n=15)*	*Group 3 (n=14)*	*Group 4 (n=15)*	$p^{\dagger}$
Systolic blood pressure (mmHg)					
Week 0	132.8±13.0	125.0±14.2	124.3±14.6	125.2±11.7	0.388
Week 8	129.2±11.1	121.0±12.2	119.6±12.9	123.8±7.9	0.175
$p^{\ddagger}$	0.319	0.208	0.112	0.581	
MD	-3.5±11.2	-3.9±11.0	-4.6±9.8	-1.3±9.0	
Diastolic blood pressure (mmHg)					
Week 0	89.9±11.1	81.1±12.3	80.4±10.5	80.0±9.5	0.105
Week 8	83.5±12.0	78.0±9.9	76.9±10.3	76.0±7.0	0.256
$p^{\ddagger}$	0.066	0.095	0.049*	0.072	
MD	-6.3±10.2	-3.1±6.5	-3.5±5.8	-4.0±7.9	
Body weight (kg)					
Week 0	78.4±16.9	71.1±9.6	70.2±10.5	71.7±10.9	0.340
Week 8	78.1±17.1	70.6±9.2	69.7±10.1	72.8±12.7	0.351
$p^{\ddagger}$	0.725	0.182	0.180	0.425	
MD	-0.2±2.3	-0.5±1.4	-0.4±1.2	1.0±5.0	
Body mass index (kg/m^2)					
Week 0	28.6±5.2	26.9±2.9	28.1±3.2	28.1±2.6	0.640
Week 8	28.5±5.4	26.7±3.1	27.9±3.1	28.5±3.3	0.565
$p^{\ddagger}$	0.793	0.242	0.203	0.421	
MD	-0.1±0.8	-0.1±0.5	-0.1±0.4	0.4±1.8	
Waist circumference (cm)					
Week 0	99.2±12.9	95.6±8.1	96.7±7.8	97.0±7.0	0.793
Week 8	99.0±12.3	92.7±9.2	93.4±7.7	94.3±6.0	0.316
$p^{\ddagger}$	0.754	0.069	0.010*	0.017*	
MD	-0.2±2.6	-2.9±5.8	-3.3±3.9	-2.6±3.7	
Fat mass (kg)					
Week 0	26.2±6.8	25.5±5.7	27.1±4.7	26.7±4.7	0.896
Week 8	22.5±7.7	24.7±6.2	26.4±5.3	26.2±4.5	0.451
$p^{\ddagger}$	0.207	0.077	0.109	0.063	
MD	-3.7±6.9	-0.8±1.5	-0.6±1.3	-0.4±0.8	
Visceral fat (%)					
Week 0	11.8±3.6	8.9±2.4	8.5±2.1	9.5±3.3	0.050
Week 8	11.7±3.5^{a}	8.6±2.2^{a,b}	8.2±2.0^{b}	9.5±3.2^{a,b}	0.024*
$p^{\ddagger}$	0.678	0.104	0.019*	1.000	
MD	-0.1±0.7	-0.2±0.5	-0.3±0.4	0.0±0.5	
Muscle mass (kg)					
Week 0	43.4±8.1	42.5±7.9	39.4±6.3	41.6±6.1	0.574
Week 8	43.7±8.0	42.9±7.9	39.6±6.2	41.8±5.6	0.523
$p^{\ddagger}$	0.223	0.136	0.053	0.321	
MD	0.3±0.7	0.3±0.9	0.2±0.3	0.1±0.6	

Group 1: PBP; group 2: WPI with cocoa powder; group 3: PBP with WPC; group 4: WPI with milk powder

Data are presented as mean±*SD*, *MD*=Mean difference.

$^{\dagger}p$-values were calculated using one-way ANOVA test.

$^{\ddagger}p$-values were calculated using paired *t*-test.

$^{a, b, c}$ Different alphabets denote significant difference between groups.

Table 2. Comparison of biochemical parameters between Week 0 and Week 8

Variables	*Group 1 (n=14)*	*Group 2 (n=15)*	*Group 3 (n=14)*	*Group 4 (n=15)*	$p^{\dagger}$
Fasting blood glucose (mmol/L)					
Week 0	5.2±0.4	5.0±0.5	5.9±3.6	5.0±1.2	0.582
Week 8	5.5±0.4	5.2±0.5	6.1±4.2	5.2±1.3	0.669
$p^{\ddagger}$	0.048*	0.002**	0.204	0.035*	
MD	0.2±0.4	0.1±0.1	0.2±0.6	0.2±0.3	
Fasting insulin (pmol/L)					
Week 0	34.5.0±27.4	55.0±33.5	53.5±36.3	48.1±30.8	0.383
Week 8	41.1±32.5	26.9±26.8	27.1±32.0	32.8±15.0	0.523
$p^{\ddagger}$	0.402	0.024*	0.016*	0.036*	
MD	6.6±26.3	-28.0±41.0	-26.3±34.0	-15.2±24.4	
HOMA-IR					
Week 0	1.4±1.0	1.9±1.0	1.9±1.2	1.5±0.4	0.477
Week 8	1.7±1.3	1.1±1.0	0.7±0.3	1.1±0.4	0.182
$p^{\ddagger}$	0.460	0.069	0.031*	0.084	
MD	0.2±1.1	-0.8±1.4	-1.2±1.4	-0.3±0.5	
Total cholesterol (mmol/L)					
Week 0	5.6±1.0	5.6±0.9	5.4±1.0	5.2±0.7	0.657
Week 8	5.3±0.9	5.1±0.7	5.3±1.0	5.0±0.6	0.776
$p^{\ddagger}$	0.310	0.007**	0.411	0.251	
MD	-0.2±0.8	-0.4±0.5	-0.1±0.5	-0.1±0.5	
Triglyceride (mmol/L)					
Week 0	1.7±0.7	1.2±0.6	1.3±0.6	1.5±0.7	0.331
Week 8	1.4±1.0	1.2±0.8	1.3±0.5	1.3±0.7	0.952
$p^{\ddagger}$	0.344	0.742	0.723	0.026*	
MD	-0.2±0.9	0.0±0.5	-0.0±0.5	-0.1±0.3	
HDL-C (mmol/L)					
Week 0	1.2±0.2	1.5±0.3	1.4±0.2	1.4±0.4	0.121
Week 8	1.2±0.2	1.5±0.3	1.4±0.2	1.4±0.3	0.124
$p^{\ddagger}$	0.467	0.924	0.896	0.344	
MD	0.0±0.1	0.0±0.0	-0.0±0.1	-0.0±0.2	
LDL-C (mmol/L)					
Week 0	3.5±0.9	3.5±1.0	3.4±0.9	3.0±0.7	0.461
Week 8	3.3±1.0	3.0±0.7	3.3±0.8	3.0±0.5	0.529
$p^{\ddagger}$	0.451	0.004**	0.556	0.867	
MD	-0.1±0.8	-0.4±0.5	-0.0±0.5	-0.0±0.5	
AST (μkat/L)					
Week 0	0.3±0.1	0.3±0.0	0.3±0.1	0.3±0.1	0.548
Week 8	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.870
$p^{\ddagger}$	0.034*	0.751	0.085	0.142	
MD	-0.0±0.0	-0.0±0.0	-0.0±0.0	-0.0±0.1	

Table 2. Comparison of biochemical parameters between Week 0 and Week 8 (continued)

Variables	*Group 1 (n=14)*	*Group 2 (n=15)*	*Group 3 (n=14)*	*Group 4 (n=15)*	$p^{\dagger}$
ALT (µkat/L)					
Week 0	0.3±0.1	0.2±0.0	0.4±0.3	0.4±0.2	0.277
Week 8	0.3±0.1	0.2±0.1	0.3±0.2	0.3±0.1	0.860
$p^{\ddagger}$	0.376	0.355	0.028*	0.176	
MD	-0.0±0.1	-0.0±0.0	-0.1±0.1	-0.0±0.1	
Blood urea nitrogen (mmol/L)					
Week 0	4.3±1.1	4.4±1.2	3.9±0.7	4.3±1.2	0.603
Week 8	5.1±1.4	4.9±1.2	4.0±1.0	4.8±1.2	0.118
$p^{\ddagger}$	0.040*	0.245	0.694	0.042*	
MD	0.8±1.2	0.4±1.4	0.1±1.1	0.4±0.8	
Creatinine (µmol/L)					
Week 0	70.8±15.6	63.7±9.8	62.0±11.7	71.8±9.3	0.079
Week 8	73.1±17.9	65.0±9.5	63.1±10.7	73.3±10.2	0.070
$p^{\ddagger}$	0.376	0.263	0.283	0.234	
MD	2.3±8.1	1.2±3.9	1.1±3.7	1.4±4.5	
Uric acid (µmol/L)					
Week 0	364.3±73.9	310.6±78.5	295.3±92.2	338.2±80.6	0.157
Week 8	391.1±87.4[a]	289.7±51.9[b]	282.5±79.7[b,c]	333.9±108.2[a,b,c]	0.008**
$p^{\ddagger}$	0.231	0.084	0.177	0.814	
MD	26.7±73.1	-20.8±41.5	-12.7±33.3	-4.3±70.5	

HOMA-IR, homeostatic model assessment of insulin resistance; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase

Group 1: PBP; group 2: WPI with cocoa powder; group 3: PBP with WPC; group 4: WPI with milk powder

Data are presented as mean±*SD*, *MD*=Mean difference

†*p*-values were calculated using one-way ANOVA test.

‡*p*-values were calculated using paired *t*-test.

[a, b, c] Different alphabets denote significant difference between groups.

significant decrease in the oxidative stress marker, malondialdehyde (MDA), (*MD*: -885.8±1044.5 nmol/ml, *p*=0.025; -750.9±979.8 nmol/ml, *p*=0.022, respectively), while MDA in the remaining groups tended to decrease.

Dietary intake of the participants

Comparing between study groups, differences were detected in energy intake, protein intake, and fat intake at week 8 (*p*=0.036, *p*=0.006, and *p*=0.002, respectively), as shown in Table 4. The dietary patterns at Week 0 and at Week 8 within group 1, group 2, and group 3 were similar (*p*>0.05 for all). However, at Week 8, group 1 and group 3 were likely to have lower energy intake, while group 2 and group 4 tended to have higher energy intake compared to pre-intervention. A significant difference within the study group was detected in group 4. After the intervention, the average fat intake of group 4 was greater than pre-intervention (*MD*: 9.7±11.7 g, *p*=0.015).

Table 3. Changes in markers of inflammation, antioxidants, and oxidative stress between Week 0 and Week 8

Variables	*Group 1 (n=14)*	*Group 2 (n=15)*	*Group 3 (n=14)*	*Group 4 (n=15)*	$p^{\dagger}$
hs-CRP (nmol/L)					
Week 0	18.7±14.9	27.8±19.4	38.1±22.7	30.6±26.8	0.200
Week 8	25.3±21.9	33.0±25.8	39.4±25.3	34.9±21.2	0.547
$p^{\ddagger}$	0.043*	0.234	0.827	0.229	
MD	6.5±9.9	5.1±14.1	1.2±18.9	4.2±11.6	
TNF-α (pg/ml)					
Week 0	198.5±189.9	94.7±52.8	318.8±238.9	222.4±225.0	0.080
Week 8	162.0±208.5	137.9±88.9	351.5±314.9	357.4±269.2	0.071
$p^{\ddagger}$	0.196	0.101	0.669	0.109	
MD	-36.5±91.9	43.1±74.6	32.7±246.2	135.0±207.8	
IL-6 (pg/ml)					
Week 0	310.2±50.9	326.6±36.9	297.0±76.2	265.4±93.7	0.156
Week 8	81.6±22.1	102.0±57.0	174.4±160.2	147.4±82.0	0.079
$p^{\ddagger}$	<0.001***	<0.001***	0.020*	0.002**	
MD	-228.6±56.4	-224.5±73.8	-122.5±173.5	-117.9±112.2	
Lactoferrin (μg/L)					
Week 0	2,266.2±409.0	2,277.5±240.7	2,309.8±433.8	2,158.7±412.1	0.758
Week 8	2,006.5±292.6[a]	1,871.0±264.6[a,b]	1,564.9±503.6[b]	1,841.0±410.5[a,b]	0.029*
$p^{\ddagger}$	0.004**	<0.001***	0.001**	0.063	
MD	-259.7±283.3	-406.5±332.4	-744.9±586.6	-317.6±557.9	
ORAC (μM TE/ml)					
Week 0	13.0±0.9	12.7±1.5	13.3±1.8	13.2±1.8	0.726
Week 8	13.3±2.2	14.4±2.1	13.9±1.7	13.8±3.6	0.755
$p^{\ddagger}$	0.641	0.026*	0.489	0.528	
MD	0.3±2.6	1.7±2.6	0.5±2.6	0.6±3.7	
MDA (nmol/ml)					
Week 0	1,994.0±726.0	2,083.6±1,172.2	2,071.7±1,161.1	1,864.9±580.0	0.940
Week 8	1,108.2±574.3	1,167.1±652.1	1,432.9±718.6	1,114.0±671.5	0.595
$p^{\ddagger}$	0.025*	0.064	0.186	0.022*	
MD	-885.8±1044.5	-916.5±1539.2	-638.7±1566.8	-750.9±979.8	

hs-CRP, high sensitivity C-reactive protein; TNF-α: tumour necrosis factor-alpha; IL-6: interleukin-6; ORAC: oxygen radical absorbance capacity; MDA, malondialdehyde
Group 1: PBP; group 2: WPI with cocoa powder; group 3: PBP with WPC; group 4: WPI with milk powder
Data are presented as mean±*SD*, *MD*=Mean difference.
†*p*-values were calculated using one-way ANOVA test.
‡*p*-values were calculated using paired *t*-test.
[a, b] Different alphabets denote significant difference between groups.

DISCUSSION

Inflammatory markers increase with fat accumulation (de Heredia, Gómez-Martínez & Marcos, 2012; WHO, 2000). An increase in these markers may interfere with the production and secretion of appetite regulating hormones such as leptin. This may result in raised appetite and probably stimulates fat accumulation, thus increasing blood lipids such as TG and LDL-C, and the risk of insulin

Table 4. Comparison of dietary intake between Week 0 and Week 8

Variables	*Group 1 (n=14)*	*Group 2 (n=15)*	*Group 3 (n=14)*	*Group 4 (n=15)*	$p^{\dagger}$
Energy intake (kcal/d)					
Week 0	1063±203	915±254	1018±227	1066±167	0.376
Week 8	1053±314	929±165	900±151	1167±257	0.036*
$p^{\ddagger}$	0.920	0.837	0.216	0.122	
MD	-10±210	14±235	-12±264	101±187	
Carbohydrate (g/d)					
Week 0	73.0±35.0	71.8±25.7	85.4±37.7	79.8±26.9	0.719
Week 8	92.5±43.0	89.8±24.9	81.1±29.4	90.3±23.4	0.830
$p^{\ddagger}$	0.096	0.073	0.601	0.300	
MD	19.5±33.2	18.0±31.5	-4.3±26.7	10.4±31.8	
Protein (g/d)					
Week 0	78.7±17.4	67.2±10.6	65.4±19.8	81.1±18.0	0.071
Week 8	82.8±17.0^{a}	72.5±8.6^{a,b}	63.6±16.6^{b}	83.2±14.7^{a}	0.006**
$p^{\ddagger}$	0.317	0.213	0.668	0.703	
MD	4.0±12.1	5.2±15.1	-1.7±14.4	2.1±15.9	
Fat (g/d)					
Week 0	43.8±15.9	33.5±13.0	29.1±4.6	42.8±16.5	0.084
Week 8	56.5±25.0^{a}	40.7±11.5^{a,b}	24.5±6.5^{b}	52.6±16.8^{a}	0.002**
$p^{\ddagger}$	0.169	0.152	0.139	0.015*	
MD	12.6±26.6	7.1±16.1	-4.6±7.1	9.7±11.7	

Group 1: PBP; group 2: WPI with cocoa powder; group 3: PBP with WPC; group 4: WPI with milk powder

Data are presented as mean±*SD*, *MD*=Mean difference.

†*p*-values were calculated using one-way ANOVA test.

‡ -values were calculated using paired *t*-test.

$^{a, b}$ Different alphabets denote significant difference between groups.

resistance, which can eventually lead to non-communicable diseases (Witard *et al.*, 2014). Whey protein is currently trendy among health-conscious people due to its advantages in maintaining a balance between muscle and fat mass. Therefore, this study aimed to evaluate the health effects of consuming 50 g of whey protein for 8 consecutive weeks in overweight and obese individuals.

The participants were likely to have reduced systolic and diastolic blood pressure after the study, especially those who consumed PBP with WPC, which significantly decreased diastolic blood pressure. Likewise, remarkable changes were also found in WC and visceral fat in the participants who consumed PBP with WPC. The effects of whey protein consumption on anthropometric parameters found in this study, especially abdominal obesity markers, were consistent with previous studies. A decrease in body weight, BMI, and WC was reported among the participants who consumed protein-fortified biscuits (total protein 50 g/day) for 8 weeks, compared to those who consumed biscuits fortified with wheat bran (Hassanzadeh-Rostamia, Abbasib & Faghiha, 2020). Similarly, a study conducted by Yang *et al.* (2014) also found a decrease in body weight, BMI, and WC in participants who consumed 30 g whey protein concentrate daily for 12 weeks. Additionally, previous studies

have also reported a decrease in fat mass and an increase in basal metabolic rate after consumption of whey protein (Zhou *et al.*, 2011; Acheson *et al.*, 2011).

About biochemical parameters, the participants who were supplemented with PBP, or WPI with cocoa powder, or WPI with milk powder, revealed an increasing trend of FBG. While intake of whey protein resulted in decrease of fasting insulin concentration, insulin tended to increase in those who consumed PBP. Regarding HOMA-IR, participants who consumed PBP with WPC saw reduced risk in insulin resistance, whereas the risk was more likely to increase in the group that consumed PBP. However, in an Australian study, obese participants had no remarkable change in serum glucose after supplementation with whey protein for 12 weeks. Nevertheless, there was a significant decrease in fasting insulin, HOMA-IR, total cholesterol, and LDL-C (Pal, Ellis & Dhaliwal, 2010). Similarly, glucose levels among hypertensive adults who consumed whey protein were also not altered, whereas insulin sensitivity was greater (Fekete *et al.*, 2018).

A meta-analysis conducted by Amirani *et al.* (2020) revealed a significant reduction in insulin and HOMA-IR, as well as blood lipids including TG, total cholesterol, and LDL-C after intake of whey protein. In this study, atherogenic blood lipids including total cholesterol and LDL-C were outstandingly reduced after consumption of WPI with cocoa powder. A significant alteration was also found in the group which received WPI with milk powder, in the form of decreased TG after the study. Dietary intake may be the reason for explaining the cause of the alterations in both anthropometric parameters and biochemical parameters. The subject in this study had no difference in dietary intake between pre- and post-intervention, except group 4 who had increased fat intake. According to Thai recommended dietary intake (Thai RDI), the subjects consumed approximately 1 g protein/kg body weight/day, which was enough for their daily requirement. Since there were no differences in the amount of dietary intake found in this study and the subjects did not report changes in their physical activities, the alterations may have been a result of the quality of protein intake.

The effects of prolonged consumption of whey protein on liver and kidney functions are still doubted. A previous study reported that whey protein supplementation decreases levels of AST and uric acid (Chen *et al.*, 2014). In this study, AST level, an indicator of liver damage, was significantly lower after ingestion of PBP. Besides, the participants who consumed PBP with WPC showed a supportive effect on the liver as the level of ALT, an enzyme that is generally released when liver cells are damaged, was remarkably reduced. However, a marker for measuring the waste products of protein metabolism in the body, BUN, was significantly increased among the participants who consumed PBP and WPI with milk powder after completing the study. A trend towards elevated BUN was also found among those who consumed WPI with cocoa powder or PBP with WPC. Therefore, the alterations probably resulted from higher protein consumption and insufficient intake of water. The interventions had no significant effects on levels of creatinine and uric acid. However, consumption of whey protein tended to lower the risk of precipitation of monosodium urate around tissues surrounding the joints and kidneys, but consumption of plant-based protein presented an increasing trend.

Although protein supplements resulted in a positive alteration of IL-6 inflammatory marker, PBP revealed a significant negative effect on inflammation by increasing levels of hs-

CRP. In addition, lactoferrin, a protein found in mammalian milk that plays a role in regulating inflammatory response by stimulating pro-inflammatory cytokine secretion, promoting the digestive tract, and preventing oxidative stress (Czosnykowska-Łukacka *et al.*, 2019; Demmelmair *et al.*, 2017; Queiroz, Assis & Júnior, 2013; Actor, Hwang & Kruzel, 2009), was decreased after intake of the given supplement. A previous study reported that after consumption of 15 g whey protein for 12 weeks, hs-CRP and IL-6 levels in overweight and obese participants were more likely to reduce (Yang *et al.*, 2019). In accordance, decreased levels of CRP, IL-6, and TNF were also presented after consuming 54 g WPI for 12 weeks (Pal & Ellis, 2010). In addition, a study also found alterations in inflammatory markers after the intake of a 60 g whey protein with 30 g high-fibre wheat bran product for 12 weeks. The study reported that TNF-α was significantly reduced, whereas hs-CRP did not alter (Rakvaag *et al.*, 2019). Similarly, there was no difference found in hs-CRP level after consumption of a high or low protein diet (Santesso *et al.*, 2012).

Regarding the effect of whey protein on antioxidant capacity, an increasing trend was found in all participants, especially among those who consumed PBP with WPC. This group had significantly elevated ORAC. Reasonably, the oxidative stress marker, malondialdehyde, was remarkably decreased after consecutively consuming PBP and WPI with milk powder. A reduced trend was also observed in those who consumed PBP with WPC or WPI with cocoa powder. However, a previous study reported that daily consumption of 40 g whey protein for 12 consecutive weeks had no effect on oxidation process, inflammatory response, and blood glucose regulation, although fat mass was significantly reduced (Flaim *et al.*, 2017).

This study had limitations on gender-based outcomes. There were no data regarding gender differences due to the small sample size in each group. Therefore, further study should include analysis and results regarding the differences between males and females.

CONCLUSION

Whey protein can potentially decrease the risk of non-communicable diseases by promoting proper fat mass and muscle mass, as well as regulating atherogenic forms of blood cholesterol. The positive effects of whey protein on health seem to be greater than that of PBP as whey protein consumption did not interfere with kidney and liver functions. Consumption of protein supplements was found to reduce the secretion of the inflammatory marker, IL-6, while PBP resulted in increased hs-CRP. In addition, antioxidant capacity was significantly higher and oxidative stress marker level was significantly decreased after intake of whey protein. Thus, proper consumption of whey protein will be beneficial to health in terms of regulating body composition and inflammation, as well as promoting antioxidant function in the body.

List of abbreviations

PBP: plant-based protein; WPI: whey protein isolate; WPC: whey protein concentrate; BMI: body mass index; hs-CRP: high sensitivity C-reactive protein; IL-6: interleukin-6; TNF-α: tumour necrosis factor-alpha; WC: waist circumference; FBG: fasting blood glucose; HOMA-IR: homeostatic model assessment of insulin resistance; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; TG: triglyceride; AST: aspartate aminotransferase; ALT: alanine aminotransferase; BUN: blood urea nitrogen; ORAC: oxygen radical absorbance capacity; MD: mean difference.

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Authors' contributions

CP, principal investigator, conceptualised and designed the study, supervised data collection, advised on data analysis and interpretation, prepared the draft of the manuscript, and reviewed the manuscript; CP, led the data collection, data analysis and interpretation, assisted in drafting of the manuscript, and reviewed the manuscript; CH, conceptualised and designed the study, advised on data analysis and interpretation, and reviewed the manuscript; PPP, led the data collection and reviewed the manuscript; KK, advised on data analysis and interpretation, and reviewed the manuscript.

Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Telomere length in Thai Buddhist monks and Thai males aged 40 years and above

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ABSTRACT

Introduction: The daily lifestyles of Thai Buddhist monks and Thai males differ due to Buddhist practices, which potentially affect telomere length. Telomeres are DNA compounds located at the ends of chromosomes that shorten with each cell division. This study investigated the difference in telomere length between Thai Buddhist monks and Thai males aged ≥40 years. **Method:** This was a cross-sectional study involving 100 Thai Buddhist monks aged ≥40 years who had been ordained for more than five years and 100 Thai males aged ≥40 years. General information and health information were assessed by questionnaire. Nutritional status was determined by body composition and blood chemistry parameters. Telomere length was measured by Monochrome Multiplex Real-Time Quantitative PCR and expressed as T/S ratio. **Result:** Mean telomere length of Thai Buddhist monks was longer than that of Thai males (1.08±0.18 vs. 1.02±0.17; p<0.050). In both groups, the mean telomere length in subjects aged ≥60 years was shorter than that in subjects aged 40-59 years (p<0.010). Alcohol consumption, which affected Thai males (p<0.050), but meditation tend to slow down the shortening of telomeres (r=0.167; p<0.050) in both groups. **Conclusion:** Age was the parameter that affected telomere length the most. Furthermore, various factors in the Buddhist monk group, such as a peaceful lifestyle, meditation, non-alcohol consumption, and fewer underlying diseases, could explain for the longer telomere lengths in this group.

Keywords: age groups, meditation, nutritional status, telomere length, Thai Buddhist monks

INTRODUCTION

Telomeres are deoxyribonucleic acid (DNA) molecules located at the end of the chromosome. They have a nucleoprotein structure, which has a repetitive nucleotide sequence. The nucleotide sequence in mammals is TTAGGG (Lu *et al.*, 2013). Telomeres are bound with telomere-specific proteins, known as telosomes or shelterin (de Lange, 2005), which protect chromosome tips from end-to-end fusion and chromosome degradation, thus increasing DNA stability. Telomeres are

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also known as chromosome caps because they resemble a plastic or metal casing at the tip of the shoelace that protects the laces from fraying. Telomere research has progressed because scientists have considered that telomeres may predict chronological ageing and slow down senescence.

Telomerase enzymes elongate telomeres and add telomeric hexanucleotide repeat sequences to the ends of chromosomes. However, telomerase is not normally active in most somatic cells, so telomeres naturally shorten with age, shrinking with each cell division until they undergo apoptosis (cell death) (Ramirez *et al.*, 2003). However, age-related diseases, such as hypertension (Bhupatiraju *et al.*, 2012) and diabetes (Olivieri *et al.*, 2009), as well as lifestyle factors, including obesity (Zannolli *et al.*, 2008) and smoking (Valdes *et al.*, 2005), can cause oxidative stress or DNA damage that accelerates telomere attrition. On the contrary, numerous studies have indicated that meditation is beneficial for one's health and affects telomere length (Alda *et al.*, 2016; Hoge *et al.*, 2013).

Thai Buddhist monks are males who have been ordained and must adhere to strict behavioural and practical rules. Their daily routine consists of alms rounds, Dharma study, prayer, and meditation. Additionally, they also adopt some special dietary habits (Ariyesako, 1998), such as eating meals before noon, after which they are not allowed to eat anything until the next morning, and not drinking alcoholic beverages. Hence, the lifestyle differences between Thai Buddhist monks and the general people, especially Thai males, are noticeable. The purpose of this study was to investigate the difference in telomere length between Thai Buddhist monks and Thai males aged ≥40 years, and to study the relationship between telomere length, nutritional status, and lifestyle factors. This study will provide new information on the effects of meditation and knowledge about the practices of Buddhist monks that may slow down telomere shortening.

MATERIALS AND METHODS

This was a cross-sectional study that was conducted with Thai Buddhist monks and Thai males; it was the first study of telomere length in Thai Buddhist monks. The inclusion criterion was age ≥40 years. Subjects who were Thai Buddhist monks had to have been ordained for more than five years. The group of Thai males was recruited from various clubs and communities close to the temples where data were collected. Thai males and Thai Buddhist monks with normal body weight and obesity were classified by percent body fat (Gallagher *et al.*, 2000). The total number of subjects was 100 Thai males and 100 Thai Buddhist monks. Those with liver disease, kidney disease, thyroid disease, or anaemia according to a screening blood examination were excluded.

General and health information

General and health information, including dietary behaviour and stress information, were collected with a questionnaire.

Body composition assessment

The parameters of body composition were height (cm), body weight (kg), body mass index (BMI, kg/m^2) and body fat (% of body weight), which were used to divide participants according to obesity and normal body weight (Gallagher *et al.*, 2000), fat mass (kg), fat-free mass (FFM, kg), muscle mass (kg), total body water (%), bone mass (kg), and visceral fat, which was used to classify normal fat and excess visceral fat levels (Tanita, 2000). These parameters were assessed using a Tanita BC-420 MA body

composition analyser (Tanita Co. Ltd., Japan).

Biochemical assessment

After a ten-hour overnight fast, fifteen millilitres of blood was collected and analysed using an automated blood BS-400 Chemistry Analyzer (Mindray Bio-Medical Electronics Co., Ltd. Shenzhen, China) for haemoglobin, fasting plasma glucose (FPG), blood urea nitrogen (BUN), and creatinine.

Telomere length measurement

DNA was purified from the sample (buffy coat) by a QIAamp DNA Blood Mini Kit (Qiagen, Germany). Telomere length was determined using the monochrome multiplex quantitative PCR (MMq-PCR) method, which evolved from the singleplex quantitative PCR assay (Cawthon, 2009). This method uses less DNA and takes less time to detect telomere length than other methods. The result of this method was the relative average telomere length, which is defined as a ratio of the quantity of telomere DNA (T) normalised to the copy number of single-copy nuclear genes (S), expressed as T/S ratio calculated per sample by CT values (T/S ratio = 2 (CT), where CT = CT sample – CT reference curve).

Ethics approval and permission

This study, including the protocols and consent forms signed by the subjects, was approved by the Human Research Ethics Committee, Faculty of Medicine Ramathibodi Hospital, Mahidol University (ID 10-61-59). Written informed consent was obtained from the subjects.

Statistical analysis

Statistical analysis was performed using SPSS statistics version 18.0. General information, health information, dietary behaviour data, stress information, nutritional status, and telomere length measurements were all expressed as mean (± standard deviation, *SD*) or percentage. The independent sample *t*-test was used to examine the difference in mean telomere length among different variables. Pearson's correlation coefficient and multiple linear regression were used to assess the relationship between telomere length and various variables. Statistical significance was defined as a *p*-value <0.05.

RESULTS

Table 1 shows that age was divided into two groups: 40-59 years and ≥60 years. The average ages of Thai males and Thai Buddhist monks were not significantly different in each age group. In both Thai males and Thai Buddhist monks, the average values for each body composition variable were not significantly different. The average fasting plasma glucose (FPG) level of Thai Buddhist monks was within the normal range (97.22±22.17 mg/dL), while Thai males had an average FPG level within the pre-diabetes range (101.62±18.21 mg/dL). However, there was no significant difference in FPG levels between the groups.

Thai male subjects had more underlying diseases (62.0%) than Thai Buddhist monks (52.0%). Hypertension, dyslipidaemia, and diabetes were the three most common underlying diseases in this study. A total of 19.0% of Thai males consumed alcoholic beverages, while Thai Buddhist monks are not allowed to consume alcoholic beverages for the duration of their ordained life. Non-smokers accounted for 75.0% and 58.0% of Thai males and Thai Buddhist monks, respectively. Most of the subjects engaged in physical activity for 3-5 days/week for 30-60 minutes/day. The percentages of subjects who added sugar to their beverages were 47.0% among Thai males and 39.0% among Thai Buddhist monks. A total of 93.0% of Thai

Table 1. Characteristics of subjects

Characteristics	*Thai males (n=100)*		*Thai Buddhist monks (n=100)*	
Age (years), mean±*SD*				
40–59 years	50.0±5.9	(*n*=50)	49.5±5.7	(*n*=62)
≥ 60 years	69.5±6.8[a]	(*n*=50)	68.5±6.8[a]	(*n*=38)
Body composition, mean±*SD*				
Weight (kg)	70.2±12.2		69.4±13.1	
BMI (kg/m^2)	25.0±4.1		25.5±4.1	
Body fat (%bw)	22.3±6.2		22.9±5.9	
Fat mass (kg)	16.2±7.1		16.6±7.3	
Visceral fat	13.2±4.6		13.2±4.3	
FPG (mg/dL), mean±*SD*	101.6±18.2		97.2±22.2	
Underlying diseases, *n* (%)				
Yes	62 (62.0)		52 (52.0)	
No	38 (38.0)		48 (48.0)	
Alcohol consumption, *n* (%)				
Drinker	19 (19.0)		-	
Non – drinker	81 (81.0)		100 (100.0)	
Smoking status, *n* (%)				
Smoker	9 (9.0)		20 (20.0)	
Ex – smoker	16 (16.0)		22 (22.0)	
Non – smoker	75 (75.0)		58 (58.0)	
Physical activity[†], *n* (%)				
Yes	74 (74.0)		82 (82.0)	
No	26 (26.0)		18 (18.0)	
Adding sugar to beverages, *n* (%)				
Yes	47 (47.0)		39 (39.0)	
No	53 (53.0)		61 (61.0)	
Meditation				
Yes	47 (47.0)		93 (93.0)	
No	53 (53.0)		7 (7.0)	

[a]Significant difference from 40–59 years, $p<0.001$
[†]Physical activity for Thai Buddhist monks: walk for alms, clean the temple yard
BMI: body mass index, FPG: fasting plasma glucose

Buddhist monks meditated, and they spent 10-30 minutes/day meditating. However, half of Thai male subjects (53.0%) did not practise meditation.

The mean relative telomere length (TL) of Thai Buddhist monks was significantly higher than that of Thai males, at 1.08±0.18 and 1.02±0.17, respectively ($p<0.05$). In both Thai males and Thai Buddhist monks, the mean TL of subjects aged 40-59 years was significantly longer than that of subjects aged ≥60 years, as shown in Table 2.

Table 3 shows the mean TL of Thai males and Thai Buddhist monks classified by body composition parameters. Thai Buddhist monks showed a higher mean TL than Thai males across all BMI categories, especially significant in obese group (I). Thai Buddhist monks showed longer TL than Thai males in all body fat

Table 2. Mean (±SD) and range of relative telomere length classified by subject group and age group

Age (years)	*T/S ratio (range)*			
	Thai males	*n*	*Thai Buddhist monks*	*n*
40-59	1.08±0.14 (0.74–1.48)	50	1.13±0.17 (0.84–1.56)	62
≥60	0.97±0.17[a] (0.70–1.48)	50	1.00±0.16[a] (0.67–1.47)	38
Total	1.02±0.17 (0.70–1.48)	100	1.08±0.18[b] (0.67–1.56)	100

[a]Significant difference from aged 40–59 years, $p<0.010$
[b]Significant difference from Thai males, $p<0.050$

percentage criteria and fat mass index groups. At both visceral fat groups, the mean TL of Thai Buddhist monks was longer than that of Thai male subjects, significantly in the groups with excess visceral fat levels. However, there was no difference in TL when comparing normal weight subjects and obese subjects.

In subjects who did not have underlying diseases, Thai Buddhist monks had a significantly higher mean TL than Thai males and Thai Buddhist

Table 3. Mean±*SD* relative telomere length classified by subject group and body composition parameters

Parameters	*T/S ratio*			
	n (%)	*Thai males*	*n (%)*	*Thai Buddhist monks*
BMI (kg/m^2)				
Normal	30 (30.0)	1.02±0.17	28 (28.0)	1.07±0.15
Overweight	19 (19.0)	1.03±0.18	22 (22.0)	1.05±0.16
Obese I	39 (39.0)	1.02±0.17	34 (34.0)	1.12±0.20[a]
Obese II	12 (12.0)	1.05±0.14	16 (16.0)	1.07±0.19
Body fat (%bw)				
Normal	53 (53.0)	1.01±0.16	53 (53.0)	1.06±0.16
Obese I	38 (38.0)	1.04±0.19	31 (31.0)	1.11±0.17
Obese II	9 (9.0)	1.02±0.12	16 (16.0)	1.08±0.23
Fat mass (kg)				
≤50th percentiles	49 (49.0)	1.02±0.16	52 (52.0)	1.06±0.15
>50th - ≤ 75th percentiles	25 (25.0)	1.03±0.19	25 (25.0)	1.12±0.21
>75th percentiles	26 (26.0)	1.03±0.17	23 (23.0)	1.09±0.20
Visceral fat level				
Normal level	37 (37.0)	1.05±0.17	39 (39.0)	1.07±0.13
Excess visceral fat level	63 (63.0)	1.01±0.17	61 (61.0)	1.09±0.20[a]

[a]Significant difference from Thai males, $p<0.050$
BMI: body mass index

Table 4. Mean±*SD* relative telomere length classified by subject group, lifestyle and health parameters

Parameters	*T/S ratio*			
	n (%)	*Thai males*	*n (%)*	*Thai Buddhist monks*
Underlying diseases				
Yes	62 (62.0)	1.03±0.19	52 (52.0)	1.03±0.16
No	38 (38.0)	1.02±0.12	48 (48.0)	1.14±0.17[a*b]
Smoking status				
Smoker	9 (9.0)	1.10±0.27	20 (20.0)	1.07±0.20
Ex – smoker	16 (16.0)	1.00±0.16	22 (22.0)	1.02±0.16[c]
Non – smoker	75 (75.0)	1.02±0.15	58 (58.0)	1.11±0.17[a**]
Adding sugar to beverages				
Yes	47 (47.0)	1.01±0.18	39 (39.0)	1.11±0.19[a***]
No	53 (53.0)	1.04±0.16	61 (61.0)	1.07±0.17
Meditation				
Yes	47 (47.0)	1.04±0.16	93 (93.0)	1.09±0.18
No	53 (53.0)	1.01±0.16	7 (7.0)	0.97±0.15
FPG				
Normal	55 (60.4)	1.06±0.17	72 (79.1)	1.09±0.18
IFG	36 (39.6)	0.98±0.14[d]	19 (20.9)	1.07±0.15[a***]

[a]Significant difference from Thai males; *p<0.001, **p<0.005,***p<0.050
[b]Significant difference from yes group; p<0.005
[c]Significant difference from non-smoker; p<0.050
[d]Significant difference from normal; p<0.050
FPG: fasting plasma glucose; IFG: impaired fasting glucose

monks who had underlying diseases. In the Thai Buddhist monk group, the average TL of subjects who were ex-smokers was significantly shorter than those who were non-smokers. Among the subjects who added sugar to their beverages, the mean TL of Thai Buddhist monks was significantly longer than that of Thai males. Furthermore, the average TL of subjects who had normal FPG was longer than subjects who had impaired fasting glucose (IFG), particularly significant in Thai male subjects. In the IFG group, Thai Buddhist monks showed significantly longer TL than Thai males. In both Thai Buddhist monks and Thai males, the average TL of those who meditated was longer than those who did not meditate. Furthermore, as shown in Table 4, Thai Buddhist monks had longer TL than Thai males in the meditation group.

Additionally, Table 5 shows that age and underlying diseases had significant negative associations with TL (r=-0.407; p<0.001 and r=-0.163; p<0.050, respectively), but meditation was found to have a significant positive association with TL (r=0.167; p<0.050) in all subjects. Age (β= -0.407, p<0.001) and alcohol consumption (β= -0.163, p<0.050) were predictor variables that influenced telomere length when age, BMI, body fat, fat mass index, visceral fat, FPG, underlying diseases, alcohol consumption, daily smoking, adding sugar in beverages, and meditation were entered as independent variables in a multiple linear regression model for telomere length.

Table 5. Pearson's correlation coefficient of relative telomere length with studied parameters

Parameters	*T/S ratio*	
	r	*p value*
Age (years)	-0.407	<0.001
Underlying diseases	-0.163	<0.050
Smoking	0.002	NS
Adding sugar to beverage	0.007	NS
Meditation	0.167	<0.050
BMI (kg/m^2)	0.046	NS
Body fat (%bw)	0.026	NS
Fat mass (kg)	0.054	NS
Visceral fat level	-0.089	NS
FPG	-0.090	NS

BMI: body mass index, FPG: fasting plasma glucose; NS: Not significant

DISCUSSION

In this study, a difference in telomere length between Thai Buddhist monks and Thai males was observed. When assessed according to BMI, especially obesity status (I), excess visceral fat, adding sugar to beverages, impaired fasting glucose (IFG) level, and meditation, Thai Buddhist monks had longer telomere length than Thai males, which could be attributed to their different lifestyles. Thai Buddhist monks leave their families and get ordained in Buddhism, where they study Dharma and follow the rules and norms of Buddhist monks. Some of their principles include no sexual intercourse, no gossiping, restriction of physical activity, which allows Buddhist monks to only walk for alms every day in the morning, perform walking meditation, and clean the temple yard as their daily movements (Agence France-Presse, 2018), and eating before noon, after which only beverages without pulp are allowed (Ariyesako, 1998). They also pray and meditate daily. These characteristics are different between Thai Buddhist monks and Thai males. As a result of these behaviours, Thai Buddhist monks may have longer telomere lengths than Thai males. When we compared telomere length in females from a previous study to telomere length in males from this study, we discovered that females had longer telomeres than males, probably due to oestrogen causing telomerase to add telomere repeats to the end of chromosomes (Nawrot *et al.*, 2004).

Telomere shortening occurs with each somatic cell replication until they reach the *Heyflick* limit (Hayflick, 1998), at which stage they finish cell replication and enter the apoptosis (cell death) process. In our study, age had an adverse correlation with telomere length, with subjects aged 40-59 years having telomere lengths longer than those aged ≥60 years. Moreover, in a previous study (Nantanawut, 2021), which investigated the difference in telomere length between individuals with normal weight and obesity in various age groups in Thai subjects, age was negatively associated with relative telomere length in both normal weight and obese groups, and other studies have shown that telomere length shortens with age (Charoenying *et al.*, 2020; Srettabunjong *et al.*, 2014). The length of telomeres is used

to calculate the biological age of the body, which is the age determined by the functioning of the body as a result of natural deterioration of the human process.

Furthermore, oxidative stress has been reported to increase in older age individuals, most likely as a result of an uncontrolled generation of free radicals induced by mitochondrial senescence and a decrease in antioxidant defences (Andriollo-Sanchez *et al.*, 2005). Oxidative stress is caused by an imbalance between antioxidants and reactive oxygen species (ROS); it occurs naturally in metabolic processes in the body or as a result of unhealthy behaviours, which can breakdown the single-stranded DNA of telomeres. Additionally, telomeres are high in guanines, which make DNA more easily oxidised and vulnerable to damage, and telomere DNA is incapable of repairing single-strand breaks, causing telomere length to shorten faster than it should (Singh *et al.*, 2019). Our research found a negative correlation between telomere length and underlying diseases, despite the weak relationship in our analysis. However, such diseases as hypertension, diabetes, and cardiovascular disease have been related to telomere shortening (Bhupatiraju, 2012.; Olivieri *et al.*, 2009.; Xu *et al.*, 2020). In addition, the average age of subjects with underlying diseases was higher than the average age of subjects without underlying diseases. As a result of telomere shortening, ageing may be one of the factors contributing to cellular senescence or age-related diseases, which induces oxidative stress and inflammation (Houben *et al.*, 2008).

In this study, Thai Buddhist monks who were non-smokers had telomere lengths longer than those who were ex-smokers, which is consistent with the results of Valdes *et al.* (2005), who reported that non-smokers had longer telomere lengths than ex-smokers. Smoking is a health risk factor that increases oxidative stress and some inflammatory markers were found to increase among ex-smokers 10-20 years after quitting (Yanbaeva *et al.*, 2007). As a result, telomere shortening may be accelerated.

Thai Buddhist monks had significantly longer telomeres than Thai males among subjects who added sugar to their beverages. While the amount of sugar added to beverages was not correlated with telomere length in this study, it was observed that the age of those who added sugar in both subject groups were significantly different, with Thai males having a significantly higher average age than Thai Buddhist monks. As a result, age may have a greater impact on telomere length in Thai males than in Thai Buddhist monks in the context of this parameter.

The study discovered that the mean telomere length in Thai males and Thai Buddhist monks with impaired fasting glucose (IFG) levels was lower than that in individuals with normal fasting plasma glucose (FPG) levels in both subject groups, which is consistent with a research that found that elderly patients with type 2 diabetes and myocardial infarction had shorter leukocyte telomere length (Olivieri *et al.*, 2009). Similarly, from the research of Sampson *et al.* (2006), who studied type 2 diabetes participants compared to control subjects, revealed that the diabetic group had a shorter mean monocyte telomere length than the control group, as well as a trend towards increased oxidative stress, which leads to telomere shortening.

We observed that alcohol consumption was one of the variables that affected telomere length in this study, similar to other studies that found that shorter telomeres were associated with alcohol consumption in older age (Dixit *et al.*, 2019) and that decreasing

telomere length was associated with increased drink units (drinking >4 drink units) (Pavenello *et al.*, 2011). Excessive drinking (five glasses in one sitting or fifteen drinks per week) produces reactive oxygen species (ROS) and increases protein, lipid, and DNA peroxidation, leading to DNA damage (Wu & Caderbaum, 2003). Alcohol consumption was only found in Thai male subjects, implying that abstaining from intoxicants that cloud the mind, such as alcohol or narcotics, was one of the precepts that Thai Buddhist monks must follow (Lin, 2017).

On the contrary, meditation can slow telomere shortening. This study found a slight positive relationship between meditation with telomere length, as over 90% of Thai Buddhist monks meditated. However, among Thai males, only approximately half practised meditation. This could be another reason why telomeres of Thai Buddhist monks were longer than those in Thai males in our study, which is in line with a study assessing telomere lengths in people from the Soto Zen Spanish Buddhist community who have practised meditation (Alda *et al.*, 2016). The study found that Zen meditators who practised meditation for more than 10 years and spent at least 60 minutes/day meditating had significantly longer telomeres than the control group (Alda *et al.*, 2016), as did a study of loving-kindness meditators, which found that the meditation group had significantly longer relative telomere length than the control group (Hoge *et al.*, 2013). Furthermore, Thai Buddhist monks were able to control their emotions more than Thai males, and they also had relatively less anxiety than Thai males. According to Buddhist doctrines that teach how to deal with anger and stress consciously, identifying the cause of the problem and resolving it effectively with wisdom and mindfulness are all related to meditation. Meditation, which focuses on the present moment, such as diaphragmatic breathing (slow and deep breathing) or loving-kindness meditation (focusing on developing a positive intention), can positively affect telomere length by reducing oxidative stress and increasing oxygen transportation to cells (Matarelli *et al.*, 2011).

CONCLUSION

Thai Buddhist monks showed longer telomere length than Thai males when the same parameters were assessed. Age was the parameter that affected telomere length the most. It was also found that underlying diseases, alcohol consumption, and meditation were related to telomere length. As a result, it is important to provide information on the advantages of meditation for self-relieving stress or problems that emerge in daily life, and the risks of drinking alcohol, which is one of the detrimental health behaviours found in the general public, except among Buddhist monks. The findings could be useful for the general public with application in health care for the prevention of telomere shortening and used as a guideline for future research. To observe telomere changes in the future, longitudinal study with larger sample sizes should be conducted. It would be interesting to study rural Buddhist monks (the Thai Forest tradition group) who place a strong focus on mental training during meditation in order to gain knowledge, they ventured out into the forest to be far away from any outside disruptions, which results in distinct daily routines than Buddhist monks who concentrate on understanding the Buddhist principles in order to share the knowledge gained with the general public, most of them stay in temples located in the city or village.

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Authors' contributions

PW, principal investigator, conceptualised and designed the study, prepared the draft of the manuscript and reviewed the manuscript; PL, conducted the study, data analysis and interpretation, assisted in drafting of the manuscript, reviewed the manuscript; AT, assisted data analysis and interpretation, reviewed the manuscript; WP, assisted in data collection and reviewed the manuscript; SB, assisted in data collection and reviewed the manuscript; NP, assisted in data collection and reviewed the manuscript.

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Conflict of interest

No conflict of interest.

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Relationship of self-regulation and lifestyle behaviour with overweight among male and female adolescents in Selangor

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ABSTRACT

Introduction: The ability of a person to self-regulate and practise healthy lifestyle behaviours determine one's weight status. The objective of this study was to determine the relationship of self-regulation, dietary practices, and physical activity with overweight status among male and female adolescents. **Methods:** This cross-sectional study used multistage cluster sampling involving government secondary school students aged between 13 and 14 years old in Hulu Langat, Selangor. Students answered a validated self-administered questionnaire comprising socio-demography, dietary practices, physical activity, and self-regulation items. Their body mass index (BMI) was calculated, and weight status was determined using the Centers for Disease Control and Prevention (CDC) BMI chart. **Results:** Among 636 students, 27.0% were "overweight", affecting more male than female students (p=0.032). Majority of them (96.7%) were motivated to maintain healthy body weight, but only a third of the students took vegetables, fruits and grains (32.4%), and performed vigorous physical activity (31.1%) regularly. Regardless of the "overweight" status, there were no significant differences in dietary practices, physical activity, and autonomous regulation for both genders. Female students showed a higher level of controlled regulation than male students in non-overweight (p=0.005) and overweight (p<0.001) groups. Higher controlled regulation increased the odds of being overweight among female students (AOR=1.04, 95% CI=1.04-1.08, p=0.010). **Conclusion:** Thus, health authorities need to develop programmes to assist, particularly female students, in practising higher autonomous regulation to combat overweight and obesity, as this group exhibits a high level of controlled regulation, which increases the likelihood of being overweight.

Keywords: school, self-regulation, students, weight status

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INTRODUCTION

Having higher than expected healthy weight is known as overweight or obesity and this affects people of all ages, including adolescents. A national survey conducted in 2019 reported that 29.8% of children aged 5 to 17 years were overweight or obese (IPH, 2020). This is alarming as evidence has shown that 8 in 10 obese adolescents will become obese adults (Simmonds *et al.*, 2016). Furthermore, if overweight or obesity is untreated, adolescents are at risk of having chronic medical conditions, such as diabetes, hypertension, and coronary heart disease, at a later age (Pell *et al.,* 2016; Bibiloni, Pons & Tur, 2015). Thus, it is crucial to curb overweight early to prevent significant morbidity and mortality in adulthood. However, overweight has no instant cure as it is strongly related to human behaviours and lifestyles.

In parallel with the rise in modernisation and industrialisation, Malaysians are experiencing lifestyle changes, including dietary practices and physical activity. Fast-food restaurants are in abundance, and new technology has made foods easily available at our fingertips. People are using online food delivery services that are available in many parts of the country because of convenience and time saving (Hooi, Leong & Yee, 2021). The advances in public transportation have eased people to move from one point to another, thus becoming less active. Understandably, inactive lifestyles and unhealthy eating behaviours have led to an increased prevalence of obesity in the population, including adolescents (Pell *et al.,* 2016). Therefore, students need to self-regulate their lifestyle to achieve ideal body weight.

Self-regulation means the ability of an individual to learn and perform goal-directed behaviours, be in control of one's emotions and behaviours, and have a good relationship with others (Pandey *et al.*, 2018). Self-regulation is an essential component for maintaining health and well-being (Pandey *et al.*, 2018). It is a continuous spectrum of self-determination, from the least to most self-determined or from amotivation to controlled (external) regulation, and subsequently autonomous (internal) regulation (Deci & Ryan, 2002). In the context of weight status, amotivation is when a person has no intention to engage in weight control practices, while controlled regulation is when a person is experiencing pressures from others in the form of guilt, praise, rewards, and punishment to maintain a normal body weight. As it does not arise from one's interest, self-determination of those with controlled regulation may be lacking, increasing the chance for failure (Deci & Ryan, 2000). On the other hand, autonomous regulation is the most self-determined state as one actively engages in activity and lifestyle to reach a normal body weight. This high level of self-determination is likely to successfully meet the goals (Deci & Ryan, 2002).

The relationship between self-regulation and weight status has been shown in cross-sectional studies among adolescents. A higher level of autonomous regulation was associated with a higher level of physical activity and hence, lower body weight (Verloigne *et al.,* 2011; Power *et al.*, 2011). On the other hand, longitudinal studies involving students with amotivation and controlled regulation showed a lower level of physical activity and the occurrence of "overweight" or "obesity" (Gropper *et al.,* 2014; Verloigne *et al.,* 2011). Thus, this self-regulation concept is an essential principle in the care of adolescents. Those who are self-motivated would engage in physical activity and hence, reach or maintain a normal weight status.

Previous local studies have highlighted that socio-demographic profiles do influence the weight status of adolescents. Being younger, male, and Malay or Indian generally have a higher prevalence of "overweight" or "obesity" (Alagappan *et al.*, 2019; IPH, 2018; IPH, 2020; Mazidi, Banach & Kengne, 2018; Pell *et al.*, 2016). Higher prevalence of "overweight" or "obesity" among male adolescents was also observed in studies conducted in European countries, such as Germany, Italy, Denmark, Hungary, as well as East Asian countries such as China and Taiwan (Wang *et al.*, 2018; Bibiloni, Pons & Tur, 2013). Healthy lifestyle behaviours are different between male and female adolescents (Mollborn, Lawrence & Hummer, 2020). In addition, female adolescents were found to be more concerned with their body weight. They showed the tendency to take a more active role in self-regulation of weight through diet and exercise compared to male adolescents (Pich *et al.*, 2015).

However, there is still a paucity of research on the relationship between lifestyle behaviours and self-regulation with body weight status. Furthermore, it is unknown whether there are any gender differences between male and female adolescents regarding lifestyle behaviours and self-regulation that would influence the "overweight" or "obesity" problem. Thus, the first aim of this study was to determine self-regulation, dietary practices, and physical activity according to body weight status among secondary school students in Hulu Langat, Selangor. Secondly, this study aimed to explore the relationship between the variables and "overweight" or "obesity" status by gender.

It is hypothesised that there could be a different level of self-regulation and lifestyle behaviours according to gender that influences "overweight" or "obesity" problem among male and female adolescents. Therefore, the findings of the present study could inform the stakeholders about the importance of empowering self-regulation of health behaviours among Malaysian adolescents to combat overweight.

MATERIALS AND METHODS

Study population

A cross-sectional study was conducted from January to March 2019 among secondary school students in Hulu Langat, Selangor. Selangor is the most developed and populated state with the highest economic growth in Malaysia. In total, there are 40 government secondary schools in six zones of the Hulu Langat district. Using the multistage cluster sampling technique, one school was randomly selected from each zone and subsequently, three to four classes of Form 1 and Form 2 were randomly selected from each school. The total number of students selected from each school was proportionate to the total number of students of the respective school. The minimum sample size required for this study was 622, with an expected 28% prevalence of overweight (Woon *et al.,* 2015), 95% confidence interval, and design effect of 2. However, considering a non-response rate of 15%, this study aimed to approach at least 715 students. All Malaysian students aged 13 to 14 years who attended the selected classrooms were invited to participate in this study. They were briefed on the study protocol, and if they agreed to participate, they were requested to get parental consent. Students without parental consent, absent or with medical illness were excluded from the study.

Study instrument

The selected students were asked to answer a Malay version self-administered questionnaire containing four sections. Section one consisted of socio-demographic questions, including

students' age, gender, ethnicity, parents' level of education, and monthly household income. The second section was the Treatment Self-Regulation Questionnaire (TSRQ) to assess the level of self-regulation (Hartmann, Dohle & Streight, 2015). The TSRQ began with a screening question, "Would you like to maintain a healthy body weight?"; those who answered 'no' or 'it does not matter to me' were grouped as not motivated or 'amotivated' and were not required to answer the remaining 14 items of TSRQ. The 14 items measured two domains of self-regulation: autonomous regulation (six items) and controlled regulation (eight items) using seven-point Likert scale responses, from 'do not agree at all' (1) to 'totally agree' (7). The expected total score for autonomous regulation ranged from 6 to 42 and 8 to 56 for controlled regulation. A higher total score indicated greater self-regulation of the assessed type. The internal consistency was excellent for the autonomous regulation domain (Cronbach's alpha=0.90) and good for the controlled regulation domain (Cronbach's alpha=0.80) (Hartmann *et al.*, 2015).

The third section was the Simple Lifestyle Indicator Questionnaire (SLIQ), which assessed the adolescents' weight-related behaviours: healthy dietary practices (three items) and physical activity level (three items). The test-retest reliability of the SLIQ was 0.63 and 0.97 (Godwinn *et al.,* 2008). In the healthy dietary practices domain, the students' frequency of eating vegetables, fruits, and grains was assessed. In contrast, the frequency of performing three types of activities (light, moderate, and vigorous) was measured in the physical activity domain. The total score of healthy dietary practices domain ranged from 0 to 15, which were then categorised into poor practice (score of 5 and less), moderate practice (score between 6 and 10), and good practice (score between 11 and 15). For the physical activity domain, the total score ranged from 0 to 9, and they were categorised into light physical activity (score of 3 and less), moderate physical activity (score between 4 and 6), and vigorous physical activity (score between 7 and 9).

Both the TSRQ (Hartmann *et al.*, 2015) and SLIQ (Godwinn *et al.,* 2008) were adapted with permission from the original authors. They were translated from English to the Malay language and *vice versa* by professional translators. These questionnaires underwent content validation by local experts and were reviewed by ten adolescents for face validity. In addition, a pilot test was conducted among 100 students from a school different from the actual study sites to determine the feasibility and reliability of the translated questionnaires**.** The internal consistency was acceptable for both TSRQ (Cronbach's alpha=0.79) and SLIQ (Cronbach's alpha=0.74).

The fourth section was on anthropometric measurements. Weight and height of the students were measured without shoes and with light clothing using a Seca 786 weighing scale with an attached stadiometer (seca GmbH, Germany), which was calibrated for each use. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). BMI was interpreted using the BMI charts by the Centers for Disease Control and Prevention (CDC) and was categorised into: underweight (BMI of <5th percentile), normal weight (BMI of between 5th to 85th percentile), overweight (BMI of between 85th to 95th percentile), and obese (BMI of >95th percentile).

This study obtained approval from the University Kebangsaan Malaysia (UKM) Ethics Committee (FF-2018-311), the Ministry of Education, the respective school principals, followed by written informed consent from

parents of students. Data were analysed using the IBM SPSS Statistics version 25.0 software (IBM Corp, Armonk, NY, USA). Descriptive analysis was presented in frequency and percentage, mean±standard deviation (*SD*), and median (interquartile range) where appropriate. Socio-demographic profiles were dichotomised into two groups. Parents were categorised as having a high level of education if they had tertiary education and low if they had secondary education or lower. Household monthly income was classified based on the Malaysian Household Income report in 2016. Parents with incomes of less than RM4000 were classified as having low income and high income if otherwise (Department of Statistics Malaysia, 2016). For overweight status, those with a BMI at or below 85th percentile were categorised as 'non-overweight', while those with a BMI of over 85th percentile were categorised as 'overweight'. Chi-square test and student *t*-test were used to determine the differences in demographic variables, lifestyle behaviours, and self-regulation according to overweight status. From these analyses, gender was the only variable associated with overweight status; therefore, subsequent analyses were stratified by gender. Multiple logistic regression (MLR) analysis with the enter method was used to identify factors associated with overweight status according to gender. The level of significance was set at $p<0.05$.

RESULTS

Out of 730 students, 636 completed the study, while 94 students were excluded due to lack of parental consent (40 students) and incomplete questionnaire (54 students), giving a response rate of 87.1%. From Table 1, among the 636 students included in the analysis, mean age of the students was 13.5±0.5 years. More than half of the students were female (53.5%), Malay (62.7%), had parents who attained secondary education (52.5%), and lived in low-income families (53.3%).

More than two-thirds of the students were non-overweight (*n*=464, 73.0%), while about one-third were overweight (*n*=172, 27.0%). Only a third of them had good dietary practices (*n*=206, 32.4%). The majority of the students reported an active lifestyle, with half of them practising moderate physical activity (326, 51.3%) and a third practising vigorous physical activity (198, 31.1%). As shown in Table 1, gender was the only significant variable associated with overweight. There were more male students in the overweight group compared to female students ($p=0.032$). Ethnicity, parental education level, family monthly income, dietary practices, physical activity, and self-regulation level were not associated with overweight.

Table 2 shows lifestyle behaviours and self-regulation by gender among non-overweight and overweight students. Female students were found to have significantly higher mean scores on controlled regulation compared to male students across both non-overweight ($p=0.005$) and overweight ($p<0.001$) groups. However, there was no difference in dietary practices, physical activity, and autonomous regulation of male and female students according to overweight status ($p>0.05$).

For questions on self-regulation, only a small proportion responded 'No' or 'It does not matter to me' to the question: 'Would you like to maintain a healthy body weight?'; thus, they were categorised as amotivated to have an ideal weight (*n*=21, 3.3%). For autonomous regulation, the expected total score ranged from 8 to 56, and the mean±*SD* total score of the students was 36.33±4.38. From Table 3, descriptive

Table 1. Socio-demographic profiles, lifestyle behaviours, and self-regulation of the students according to weight status (*N*=636)

Variables	*All*	*Non-overweight*	*Overweight*	χ^2/ *t-test*	*p-value*
Socio-demographic profiles, *n* (%)					
Gender					
Male	296 (46.5)	204 (68.9)	92 (31.1)	4.57	0.032
Female	340 (53.5)	260 (76.5)	80 (23.5)		
Ethnicity					
Malay	399 (62.7)	298 (74.7)	101 (25.3)	1.63	0.202
Non-Malay	237 (37.3)	166 (70.0)	71 (30.0)		
Father's education level					
Secondary & below	372 (58.5)	269 (72.3)	103 (27.7)	0.19	0.664
Tertiary	264 (41.5)	195 (73.9)	69 (26.1)		
Mother's education level					
Secondary & below	361 (56.8)	267 (74.0)	94 (26.0)	0.43	0.513
Tertiary	275 (43.2)	197 (71.6)	78 (28.4)		
Family monthly income					
<RM4000	339 (53.3)	253 (74.6)	86 (25.4)	1.03	0.310
>RM4000	297 (46.7)	211 (71.0)	86 (29.0)		
Lifestyle behaviours, *n* (%)					
Dietary practices					
Poor	81 (12.7)	59 (72.8)	22 (27.2)	4.171	0.124
Moderate	349 (54.9)	265 (75.9)	84 (24.1)		
Good	206 (32.4)	140 (68.0)	66 (32.0)		
Physical activity					
Light	112 (17.6)	84 (75.0)	28 (25.0)	4.75	0.093
Moderate	326 (51.3)	226 (69.3)	100 (30.7)		
Vigorous	198 (31.1)	154 (77.8)	44 (22.2)		
Self-regulation status, mean±*SD*					
Autonomous	36.33±4.38	36.47±4.21	35.96±4.82	0.70	0.202
Controlled	36.39±9.22	36.30±8.88	36.63±10.10	0.01	0.696

analysis of all the six autonomous regulation items showed no significant gender difference in terms of students' responses to each item (p>0.05), except for the item on making decision. In general, both male and female students agreed it was essential to be healthy. Moreover, they were responsible for owning health, indicating they had inner self-motivation to maintain body weight.

Table 2. Differences in lifestyle behaviours and self-regulation by gender among non-overweight and overweight adolescents (*N*=636)

Variables	*Non-overweight*		*p-value*	*Overweight*		*p-value*
	Male	*Female*		*Male*	*Female*	
Dietary practices, *n* (%)						
Poor	26 (44.1)	33 (55.9)	0.949	9 (40.9)	13 (59.1)	0.398
Moderate	118 (44.5)	147 (55.5)		45 (53.6)	39 (46.4)	
Good	60 (42.9)	80 (57.1)		38 (57.6)	28 (42.4)	
Physical activity, *n* (%)						
Light	37 (44.0)	47 (56.0)	0.998	16 (57.1)	12 (42.9)	0.550
Moderate	99 (43.8)	127 (56.2)		50 (50.0)	50 (50.0)	
Vigorous	68 (44.2)	86 (55.8)		26 (59.1)	18 (40.9)	
Self-regulation, mean±*SD*						
Autonomous	36.30±3.89	36.60±4.43	0.443	35.39±5.43	36.62±3.95	0.102
Controlled	34.96±8.76	37.32±8.85	0.005	33.53±9.33	40.17±9.83	<0.001

*Chi-square test and student *t*-test
* *p<0.05* is significant

However, more female students agreed that it was an important decision to maintain a healthy weight compared to male students (p=0.046).

For controlled regulation, the expected total score ranged from 10 to 42, and the mean±*SD* total score of the students was 36.39±9.22. From Table 4, descriptive analysis of all the eight items of controlled regulation showed more female students would be embarrassed (p<0.001), felt bad about themselves (p=0.002), felt guilty (p=0.001), and felt indiscipline (p<0.001) if they did not have a healthy body, compared with male students.

Table 5 shows the multivariate regression analysis on factors associated with overweight status according to gender. The only significant variable was female students, and higher controlled regulation was associated with overweight problem (AOR =1.04, 95% CI= 1.01,1.08, p=0.010). Other variables were not significant (p>0.05).

DISCUSSION

The prevalence of the "overweight" group among secondary school students was about a quarter (27.0%). This combined prevalence was nearly identical to that observed in a national survey conducted by the Institute for Public Health (IPH), which indicated that 29.8% of Malaysian students were overweight (IPH, 2020). However, this combined prevalence of overweight was higher than a prevalence involving overweight students from other Asian countries (23.2%). This overweight problem is alarming and calls for immediate intervention. Furthermore, the proportion of overweight or obesity was significantly higher in male students compared to female students. This finding was also evident among students in European and other Asian countries (Wang *et al.*, 2018, Mazidi *et al.*, 2018; Pell *et al.*, 2016, Bibiloni *et al.*, 2015). The possible explanation for this difference could be due to female students desiring a slimmer body. Hence, they may adopt unhealthy eating behaviours such as dieting to lose weight (Senín-calderón *et al.*, 2017, Pich *et al.,* 2015).

There was no significant relationship between ethnicity, parental education level, and family monthly income among overweight male and female students. In

Table 3. Detailed analysis of the self-regulation items by overweight status: Autonomous self-regulation (*N*=636)

Autonomous self-regulation	*Disagree*		*Neutral*		*Agree*		*p-value*
	Male n (%)	*Female n (%)*	*Male n (%)*	*Female n (%)*	*Male n (%)*	*Female n (%)*	
It is very important to be as healthy as possible	6 (75.0)	2 (25.0)	5 (33.3)	10 (66.7)	285 (46.5)	328 (53.5)	0.161
I personally believe that it is best for my health	6 (66.7)	3 (33.3)	6 (40.0)	9 (60.0)	284 (46.4)	328 (53.6)	0.422
I would like to take responsibility of my own health	3 (60.0)	2 (40.0)	16 (47.1)	18 (52.9)	277 (46.4)	320 (53.6)	0.830
It is an important decision I really want to take	7 (46.7)	8 (53.3)	46 (59.7)	31 (40.3)	243 (44.7)	301 (55.3)	0.046
I have thought about it carefully and think that this is important for many aspects of my life	7 (58.3)	5 41.7)	29 (48.3)	31 (51.7)	260 (46.1)	304 (53.9)	0.673
It fits my life goals	12 (46.2)	14 (53.8)	49 (48.0)	53 (52.0)	235 (46.3)	273 (53.7)	0.947

*Chi-square and student *t*-test
**p* value <0.05 is significant

Table 4. Detailed analysis of the self-regulation items by gender: Controlled self-regulation (*N*=636)

Controlled self- regulation	*Disagree*		*Neutral*		*Agree*		*p-value*
	Male n (%)	*Female n (%)*	*Male n (%)*	*Female n (%)*	*Male n (%)*	*Female n (%)*	
I would be embarrassed if I did not have a healthy body	80 (64.0)	45 (36.0)	47 (49.0)	49 (51.0)	169 (40.7)	246 (59.3)	<0.001
I would feel bad about myself if I do not have a healthy body	104 (57.5)	77 (42.5)	54 (44.6)	67 (55.4)	138 (41.3)	196 (58.7)	0.002
I would have a guilty conscience if I do not have a healthy body	92 (59.7)	62 (40.3)	53 (43.1)	70 (56.9)	151 (42.1)	208 (57.9)	0.001
I feel indiscipline when I do not have a healthy body	118 (58.4)	84 (41.6)	66 (46.5)	76 (53.5)	112 (38.4)	180 (61.6)	<0.001
I permanently feel pressured by others to have a healthy body weight	113 (49.1)	117 (50.9)	74 (49.0)	77 (51.0)	109 (42.7)	146 (57.3)	0.291
Others would be upset with me if I do not have healthy body weight	161 (48.2)	173 (51.8)	74 (44.8)	91 (55.2)	61 (44.5)	76 (55.5)	0.675
I want others to see that I can do it	31 (51.7)	39 (48.3)	41 (51.9)	38 (48.1)	224 (45.1)	263 (54.9)	0.372
I want others to accept me	28 (44.4)	35 (55.6)	43 (44.8)	53 (55.2)	225 (47.2)	252 (52.8)	0.858

*Chi square test
**p* value <0.05 is significant

Table 5. Logistic regression of socio-demographic profiles, lifestyle behaviours, and self-regulation with overweight among male and female students (*N*=636)

Variable	*Male (n=296)*					*Female (n=340)*				
	B	*SE*	*Wald*	*AOR*	*95% CI*	*B*	*SE*	*Wald*	*AOR*	*95% CI*
Age	-0.22	0.26	0.7	0.81	0.48-1.33	-0.18	0.27	0.44	0.84	0.50-1.41
Ethnicity: Malay [non-Malay]	-0.12	0.30	0.16	0.89	0.49-1.58	-0.38	0.29	1.65	0.69	0.39-1.22
Father's education level: High education [low education]	0.11	0.36	0.85	1.11	0.55-2.24	-0.31	0.35	0.76	0.74	0.37-1.47
Mother's education level: High education [low education]	0.22	0.35	0.39	1.24	0.63-2.45	0.46	0.35	1.73	1.58	0.80-3.11
Family monthly income: High income [low income]	0.49	0.36	0.19	1.05	0.52-2.10	-0.49	0.43	1.29	0.61	0.26 -1.43
Dietary practices: Moderate diet [poor diet]	0.15	0.44	0.12	1.16	0.49-2.73	-0.34	0.40	0.75	0.71	0.33-1.54
Good diet [poor diet]	0.72	0.47	2.34	2.26	0.82-5.18	0.36	0.43	0.01	1.04	0.44-2.42
Exercise level Moderate [light exercise]	0.15	0.44	0.12	1.16	0.49-2.73	0.47	0.38	1.49	1.59	0.75-3.36
Vigorous [light exercise]	-0.35	0.40	0.73	0.71	0.32-1.56	-0.14	0.44	0.10	0.87	0.37-2.07
Autonomous regulation	0.003	0.19	0.19	1.003	0.97-1.04	-0.05	0.03	3.23	0.96	0.91-1.00
Controlled regulation	-0.005	0.15	0.12	1.00	0.97-1.04	0.04	0.02	6.72	1.04	1.04-1.08*

*p=0.010

contrast, earlier studies have reported that overweight and obesity problems were more common among students from higher socioeconomic status, having parents with higher education levels, and a higher monthly income, as well as living in urban areas (Mistry & Puthussery, 2015, Okour *et al.*, 2019). However, this is inconsistent as recent national data showed that overweight was also prevalent in adolescents from low-income families (B40). At the same time, obesity was prevalent in adolescents from high-income families (T20) (IPH, 2020). This means that overweight and obesity affect both rich and poor Malaysian students. Thus, prevention strategies should target adolescents of all socioeconomic statuses to reduce the prevalence of overweight and obesity.

For lifestyle behaviours, only a third of the students practised a well-balanced diet containing vegetables, fruits, and grains four to six times per week. A similar finding was also observed in the National Health and Morbidity Survey 2019 that reported low daily consumption of fruits and vegetables among the Malaysian population (IPH, 2020). It is quite common for Malaysian students to have snacks and high carbohydrate fatty meals (Mohammadi *et al.*, 2019). Only a third of the students read the food label, with half of them, especially obese students, skipping breakfast and a third had carbonated soft drinks daily (IPH, 2018). This is further concerning because our current study found no difference in dietary practices between male and female students in the overweight and non-overweight groups.

In contrast to this, a systematic review showed Malaysian male students tend to have poorer diet quality and consumed foods greater in energy density and macronutrients than female students (Mohammadi *et al.*, 2019). The inconsistent findings may be due to the different age groups of adolescents focused upon in previous studies, which makes comparison of findings equivocal. Another possible explanation could be younger male and female students of 13 to 14 years old not having very much freedom to choose their foods as parents exert a strong protective influence on food choices. Therefore, they just eat anything prepared for them (Gunther *et al.*, 2019).

As for physical activity, only a third of the students performed vigorous activities, such as running, sports, and weight-lifting, more than four to seven times a week. This is far from the recommended level of at least 60 minutes of moderate to vigorous physical activity daily by the World Health Organization (WHO) (Guthold *et al.*, 2020). This low level of physical activity is a known phenomenon affecting adolescents worldwide. It was reported in a pooled analysis involving 141 countries that more than 80% of adolescents were not physically active daily (Guthold *et al.*, 2020). This study also showed no difference in the physical activity of overweight and non-overweight adolescents of both genders. The potential explanation for this includes other confounding factors that influence the weight status of adolescents and self-reporting of physical activity that may have led to various biases.

Irrespective of their weight status, majority (96.9 %) of the students were motivated to have a normal body weight. This could be attributed to their adolescence stage, where body image is essential to growing adolescents (Senín-Calderón *et al.*, 2017). The students showed a high total mean score for both autonomous and controlled regulations. However, based on the self-regulation theory, for ideal body weight, one's autonomous regulation should be higher than controlled regulation (Deci & Ryan, 2002). Looking into the detailed analysis of the autonomous regulation items,

both male and female students agreed it was vital to be healthy and take charge of their lives. Their motivation to maintain a healthy body weight was from inner-self, the students wanting to do it for their own good. However, there was no difference in the autonomous regulation of overweight and non-overweight adolescents of both genders.

Looking into the controlled regulation domain in this study, female students showed significantly higher scores on controlled regulation compared to male students in both overweight and non-overweight groups; and this higher level of controlled (external) regulation increased the odds for risk of overweight problem. This study was unique as it revealed that non-overweight females were also in danger of becoming obese. A higher number of female students would feel embarrassed, bad, guilty, or indisciplined if they did not have a healthy body weight. However, this external pressure was insufficient to make them lose weight, and they gained weight instead. This is consistent with a study among the United States adolescents that showed overweight adolescents, especially females, were associated with higher controlled regulation than normal-weight adolescents (Gropper *et al.,* 2014). The possible explanation is that female students are more concerned with their body image and self-appearance (Senin-Calderón *et al.*, 2017). As a result, people's perception and expectations (controlled regulation) can greatly affect their motivation to have an ideal weight, as opposed to male adolescents. However, the over-dependency on others' perceptions and expectations is less likely to make female adolescents sustain the motivation to achieve an ideal weight. Their impulse to change due to external pressure is temporary and requires many sacrifices. When perceiving behavioural change, females tend to break their dietary regimes and efforts to dietary practices, which is ineffective for the long term and thus, makes them prone to be overweight (Poraj-Weder, Wąsowicz & Pasternak, 2021).

An important inference that one can make from the above findings is that overweight students from both genders need intervention to increase autonomous regulation for long term weight control management. Theoretically, it is crucial for students to have high internal regulation and autonomous motivation to reduce and sustain normal weight. The greater the self-motivation, the higher the level of effort, such as engaging in physical activity, that will be given to reduce their weight (Aleksovska-Velickovska, Gontarev & Ruzdija, 2019); while failure to self-regulate may lead to obesity (Stoeckel *et al.*, 2017). Therefore, it is high time for the country to correct this, conducting more motivational programmes, and thus, making adolescents, especially those with overweight problems, have their own desire and self-motivation, and not be dependent on others to have a healthy body weight. Personal skills intervention using modelling behaviour, play therapy, and attention training may be considered as evidence has shown its efficacy in improving adolescents' self-regulation (Pandey *et al.*, 2018). Promoting self-motivation or autonomous (internal) regulation is likely to give a long-lasting commitment to weight control behaviours that include healthy dietary practices and physical activity (Hartmann *et al.*, 2015).

There are a few limitations in this study. The main limitation of a cross-sectional study is its inability to determine causal relationships between the variables. A longitudinal study would be beneficial to further understand the relationships between weight status, self-regulation, and weight-related behaviours. In addition, the use of self-

administered questionnaires could lead to socially desirable biases (Woon *et al.,* 2015), not reflecting their true motivation to maintain an ideal body weight or true dietary practices. A qualitative approach may be beneficial to explore further, especially on their internal or autonomous regulation in relation to external or controlled regulation, as well as a more detailed description of their dietary intakes. Findings from the present study should not be generalised to the whole adolescent population as this study was conducted among secondary school adolescents in the Hulu Langat district. Despite these limitations, the study also had its own strengths. The sample recruited for this study was robust using the multistage cluster sampling technique with a good response rate. The BMI and weight status of the students were based on the measurements done by the researcher and not self-reported, ensuring the reliability of the values reported. This study used well-validated questionnaires with good internal consistencies. Therefore, conclusive and reliable results were obtained, and several definite conclusions could be made.

CONCLUSION

In conclusion, the problem of overweight and obesity were highly prevalent among adolescents aged 13-14 years in Hulu Langat, Selangor, affecting more males compared to females. This study confirmed that gender influences weight status and self-regulation of one's weight status. Female students were found to have higher external (controlled) regulation compared to male students in both overweight and non-overweight groups. The feeling of embarrassment, bad, guilty, and indiscipline due to not having a healthy body had significantly influenced their weight status. The high level of controlled regulation increased the likelihood of overweight problem in female students, but not male students.

Based on these findings, intervention programmes involving both male and female students will be required to combat overweight and obesity. In addition, behavioural intervention is needed to improve self-regulation in female adolescents, particularly through motivational programmes aimed at increasing internal (autonomous) regulation over external (controlled) regulation.

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Authors' contributions

AG, site investigator, conducted the study, conceptualised and designed the study, prepared the draft of the manuscript, and reviewed the manuscript; NAM, principal investigator, conceptualised and designed the study, supervised the data collection, advised on data analysis and interpretation, and reviewed the manuscript; GR, led the data collection in schools and reviewed the manuscript; HT, advised on data analysis and interpretation, assisted in drafting of the manuscript, and reviewed the manuscript; MSH, advised on data analysis and interpretation, assisted in drafting of the manuscript, and reviewed the manuscript; KO, advised on data analysis and interpretation, assisted in drafting of the manuscript, and reviewed the manuscript.

Conflict interest

The authors declare no conflict of interest.

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Developing and pre-testing of nutrition cartoon video to promote healthy eating among hearing and deaf and mute children

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ABSTRACT

Introduction: A six-minute nutrition cartoon video "The Magical *Pinggang Pinoy* in *Nutrilandia*" was developed and pre-tested to encourage hearing and deaf and mute children to eat a variety of foods by following the *Pinggang Pinoy*® (Healthy Plate). This study described the development process of the nutrition cartoon video and explored the participants' acceptance towards it. **Methods:** The video underwent two levels of pre-testing to ensure comprehensibility, attractiveness, acceptability, and self-involvement. The first level was conducted among three DOST-FNRI experts, while the second level was among six deaf-mute school teachers and 30 mothers/ caregivers of 6-9 years old hearing children. Data were collected through an online self-administered questionnaire. Open-ended questions allowed participants to express themselves freely on the given subjects. Data analysis used thematic analysis. **Results:** The video conveyed clear information on the *Pinggang Pinoy*®, and the inclusion of animation, subtitles, visuals, and voice-over made the video easier to understand. Participants stated that the message of the video was directed to children, teens, adults, malnourished people, and everyone in general. Pre-testing the nutrition cartoon video before final production identified terminologies and concepts that participants found unfamiliar, confusing and unacceptable; offered suggestions for improvement and made pre-tested video appropriate for hearing and deaf-mute children. **Conclusion**: Overall, the participants had positive perceptions on the nutrition cartoon video. The video can be used in nutrition education classes among hearing and deaf and mute children, and serves as a tool to measure children's nutrition knowledge on healthy eating.

Keywords: cartoon video, healthy eating, hearing and deaf and mute children, nutrition education, pre-testing

INTRODUCTION

Nutrition education is any combination of educational strategies, accompanied by environmental support, designed to facilitate voluntary adoption of food choices and other food- and nutrition-related behaviours conducive to health and well-being (Contento, 2007). Thus, nutrition education programme can be developed for early childhood education

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centres (Kim & Kim, 2014). Young children face rapid development like cognitive and physical developments. Therefore, good nutrition at this stage is essential for good health, growth and development.

Nutrition education uses various strategies and approaches. One such approach uses information, education, and communication (IEC) as a tool to empower people to make decisions, modify behaviours, and change social conditions. The IEC channels include interpersonal communication, such as individual or group discussions, and community meetings and events, or mass media communication like radio, television and other forms of one-way communication, such as brochures, leaflets and posters, visual and audio-visual presentations, and some forms of electronic communication.

The Department of Science and Technology-Food and Nutrition Research Institute (DOST-FNRI) develops IEC materials as educational intervention to help change or reinforce nutrition-related behaviours among specific target audiences. This is because eating abilities and needs of children are dependent on their cognitive and social development in the first few years of life (Tremblay *et al.*, 2013). These IEC materials serve as strategic tools in helping to achieve one of DOST-FNRI's mandates, which is to diffuse knowledge and technologies in food and nutrition, and provide science and technology services to stakeholders.

A new food and nutrition IEC initiative of the Institute is the nutrition cartoon "*Ang Mahiwagang Pinggang Pinoy ng Nutrilandia*" that encourages hearing and deaf and mute children to eat a variety of foods by following the *Pinggang Pinoy*® (Healthy Plate). Cartoon shows for children are generally recommended by psychology experts. Martzoukou (2020) suggests exposing children to interactive cartoon-based activities to help them further explore and understand their perspectives about certain subjects.

Deaf and mute children are visual learners (Rosal & Echaure, 2021). The inclusion of sign-language interpretation in a cartoon video will help deaf and mute children to understand the lesson it conveys. Communication technologies, such as cartoon videos, can aid and accelerate the adoption of health education initiatives that are long-lasting, accessible, and effective to deaf and mute children (Abbasi *et al.*, 2017). Thus, both hearing and deaf and mute children can benefit from an educational cartoon video. Likewise, addressing nutritional problems among deaf and mute children early on with the aid of correct nutrition IEC strategies is encouraged.

The digital age has enabled easier access to cartoons using social media platforms. In an exploratory study by Khalid, Meerah & Halim in 2010, more than 70% of respondents had positive views on the use of cartoons in teaching and learning. Cartoons can be used as a low-cost and feasible educational tool to overcome the barriers of communication efforts among teachers and parents of deaf children (Doichinova & Peneva, 2013).

The COVID-19 pandemic has escalated the practice of blended learning or use of information and communication (ICT) in education worldwide (Bordoloi, Das & Das, 2021). ICT is a vital facet in promoting progress in the language experience of students with hearing impairments "without being dependent on the spoken words and by adopting specialised tools used either in the classroom or at a distance" (Bouzid & Jemni, 2017), such as digital video. Puspaningtyas & Ulfa (2021) mentioned that students' motivation and creative thinking abilities are enhanced through animated video in blended learning.

Health education videos with local languages were found effective in improving knowledge, attitudes, and practice of health among areas least attended by health services (Mutanda, Waiswa & Namutamba, 2016). Thus, the use of video as a learning instrument may facilitate the development of new behaviours (Tuong *et al.*, 2014). Cartoons packaged in video format with health and nutrition messages may provide an option for blended learning. Therefore, the appropriateness of cartoons as an IEC material should be established first by determining its acceptance, perception, attitude, intention, and behaviour among educators (Ibili & Sahin, 2016).

Pre-testing measures the effectiveness of common media and an important step in materials development. It allows the evaluation of messages and materials as to acceptability and potential impact before large amounts of resources are used in production and distribution. It also ensures that materials are effective, comprehensible, and persuasive (Francisco, Manlulu & Vargas, 2021).

The present study was undertaken to describe the development process of the nutrition cartoon video, explore the participants' acceptance of the nutrition cartoon video, and identify recommendations of study participants for the nutrition cartoon video through pre-testing to be more effective to its target audience.

MATERIALS AND METHODS

This was a descriptive study that discussed the development and pre-testing of a nutrition cartoon video to promote healthy eating among hearing and deaf and mute children.

Development process of the nutrition cartoon video

Nutrition educators developed the storyboard of the video in Filipino. The storyboard was based on the concept of healthy plate for Filipinos or *Pinggang Pinoy*®. *Pinggang Pinoy*® is an easy-to-understand food guide using a familiar food plate model to convey the right food group proportions on a per-meal basis to meet the body's energy and nutrient needs of adults. The storyboard of the video was reviewed by experts in the field of nutrition education and communication for accuracy of technical content. Upon approval of the storyboard, the team met with the contracted video developer to discuss the storyboard. The nutrition cartoon video underwent two levels of pre-testing to ensure that the video had good comprehensibility, attractiveness, acceptability, and self-involvement. The first level of pre-testing was done among three DOST-FNRI experts for review of technical content, while the second level was among six teachers from a deaf-mute school and 30 mothers and caregivers of 6-9 years old hearing children. Revisions were made to the video before it was pre-tested for the second level.

The nutrition cartoon video *Ang Mahiwagang Pinggang Pinoy ng Nutrilandia* encourages children to eat a variety of foods by following the *Pinggang Pinoy*® (Healthy Plate). It is six minutes in length featuring the fictional story of siblings, Akiles and Arya and their journey to *Nutrilandia*. The unique thing about the video is that it is intended for hearing and non-hearing children. The video includes an inset showing a deaf and mute translator who acted as the sign language interpreter.

Pre-testing of nutrition cartoon

The video was pre-tested among intended audience to determine whether they understood the content of the video and accepted the video before its final production.

In this study, participants were selected through purposive, snowball

sampling technique, utilising an existing list of mothers and caregivers who attended the DOST-FNRI food and nutrition training. The pre-testing was conducted among mothers of normal, hearing children aged 6-9 years old. The experts involved in the pre-testing were teachers from a deaf-mute school in the Philippines.

Pre-testing participants were recruited by sending letter of invitation via email. Interested participants were assessed based on the inclusion criteria, namely mother or caregiver and teacher with a child aged 6 to 12 years old, can read and write, can answer Google form, and with internet access. A total of 30 mothers and caregivers and six (6) experts from one of the deaf-mute institutions in the Philippines joined the study.

The instrument used in this study was a pre-tested self-administered questionnaire on attractiveness, comprehensibility, acceptability, and self-involvement that was administered online. The online questionnaire was derived from a previously developed questionnaire for pre-testing IEC materials and converted into online format in Google form. Prior to the use of the online self-administered questionnaire, a trial was conducted among selected DOST-FNRI staffs to check the comprehensibility and layout of the questionnaire in Google form. The self-administered questionnaire included instructions to first watch the nutrition cartoon video before accomplishing the online questionnaire, and informed consent and confidentially. The questionnaire included the following items: three (3) for comprehensibility; six (6) for attractiveness; four (4) for acceptability; three (3) for self-involvement, and one (1) for overall impression of the video. The open-ended questions allowed study participants to express themselves freely on the given subject.

The researcher checked thoroughly the accomplished Google form to ensure completeness of responses of the study participants. After accomplishing the online questionnaire, the study participants received PHP 300.00 (6 USD) as a token of appreciation for joining the study.

Responses from the open-ended questions were automatically recorded through Google form, and the matrices of data were prepared. Thematic analysis (TA) was used to present the results. TA (Braun & Clarke, 2006) is an accessible, flexible, and increasingly popular method of qualitative data analysis. Six steps of TA, as suggested by Braun and Clarke (2006) were adopted as follows: (1) familiarisation with the data; (2) generating initial codes; (3) searching for themes; (4) reviewing the themes; (5) defining and naming the themes; and (6) producing the report.

Ethical consideration

Prior to pre-testing of the nutrition cartoon, the questionnaire and informed consent form used for IEC materials were approved by the FNRI Institutional Ethics Review Committee. Information regarding the pre-testing's objectives, type of research intervention, participant selection, voluntary participation, procedure, duration, risks and benefits, reimbursements, confidentiality, sharing of results, right to refuse or withdraw, and contact details were included in the Informed Consent Form. Participants signed the consent forms and were assured of the confidentiality of information collected in the study.

RESULTS

The DOST-FNRI's six-minute nutrition cartoon "*Ang Mahiwagang Pinggang*

Pinoy ng Nutrilandia" centred on the characters of Akiles and Arya – siblings who went on a dream adventure to the *Nutrilandia* kingdom where the magical *Pinggang Pinoy* originated. However, upon their arrival, the siblings found that the people under the cruel rule of *Haring Amon* became malnourished due to inadequate food intake. The magical *Pinggang Pinoy* used to be the kingdom's food guide until *Haring Amon* destroyed it and hid the pieces in several places in the kingdom. Their mission was to find the missing pieces of *Pinggang Pinoy* in *Nutrilandia*.

The video is intended for children aged 6-9 years old, but the pre-testing was done among mothers or caregivers of 6-9 years old hearing children. The respondents in this study composed of six (6) female sign language experts from the Philippine School for the Deaf (PSD). Their ages ranged between 26-56 years old. A total of 30 mothers and caregivers from a mix of rural and urban areas were also involved in this study. Their ages ranged between 22-61 years old, consisting of two males and 28 females.

The comprehensibility, attractiveness, acceptability, and self-involvement of the IEC material were evaluated, and statements provided from the online self-administered questionnaire on each aspect can be found in Tables 1-5.

A. Perceived comprehensibility of the video

The video is comprehensible when the message is clearly understood.

A total of four (4) sign language experts and ten (10) mothers and caregivers said that the nutrition cartoon was about *Pinggang Pinoy*®. Participants also stated that the video was about Go, Grow and Glow foods, nutrition awareness, importance of eating nutritious foods to achieve a healthy body and mind, and nutrition messages and moral lessons for kids.

On the other hand, three (3) of the sign language experts said that they did not find words or sentences difficult to understand. Three (3) experts found that the sign language interpreter's emotion was sometimes not synchronised with the animation of the video, that was why they found it hard to understand the dialogues. About twenty-seven (27) mothers and caregivers said that they did not find words or sentences difficult

Table 1. Perceptions of sign language experts, mothers and caregivers on the comprehensibility of the nutrition cartoon video

Pre-testing aspect	*Theme*	*Sub theme*
Comprehensibility	What is the video all about?	The story is about *Pinggang Pinoy*
		The story is about Go, Grow, Glow
		The story serves as a guide in eating nutritious foods to achieve healthy body and mind
		The story is about the food pyramid
		Nutrition messages and or moral lesson
	Words or sentences difficult to understand in the video	Sign language sentence construction
		The words and sentences should depend upon the age of the targeted population
	Unfamiliar words or sentences in the video	The term *Pinggang Pinoy*®
		The word *magpahilan,* which means not to sleep with a full stomach so he or she will not get indigestion

Table 2. Perceptions of sign language experts, mothers and caregivers on the attractiveness of the nutrition cartoon video

Pre-testing aspect	*Theme*	*Sub theme*
Attractiveness	Things that caught your attention in this video	Beautiful illustrations and animation
		Attractive colours of the characters
		Beautiful sign language interpretation
		Lesson of the story
		Sounds and voice over
		Title of the video
		The term *Pinggang Pinoy*®
		Characters in the story
		The use of Filipino language
		Its educational value
	Reason/s for liking the appearance of the video	Attractive visuals and illustrations
		Catchy colours of the settings and characters
		Lesson of the story
		Sign language interpreter
		Awesome animation and voice over
	Reason/s for disliking the appearance of the video	Unnatural movements of the characters
		Not enough space allotted for the sign language interpreter
		The sign language interpreter covered an image in the video
		The sign language interpreter lacks emotion
		The lack of background music

to understand. Only two (2) mothers/caregivers mentioned that *Pinggang Pinoy*® was difficult to understand. One (1) respondent commented that words and sentences should depend on the age of the targeted population.

A total of five (5) sign language experts did not find unfamiliar words or sentences. Only one expert said that he was not familiar with the Filipino word *magpahilan,* which means not to sleep with a full stomach so he or she will not get indigestion. One respondent from mothers and caregivers stated that she was not familiar with *Pinggang Pinoy*® and *magpahilan.*

B. Perceived attractiveness of the video

The video is attractive when the message is interesting enough to attract and hold the attention of the target audience.

Among the sign language experts, two (2) stated that the first thing that caught their attention was the illustration, one (1) stated colours, and two (2) stated sign language interpreter. However, one (1) specified that the sign language interpreter who was positioned on the lower right corner was not lively.

Among mothers and caregivers, seven (7) stated that the first thing that caught their attention was the story or the lesson, then six (6) stated colours,

Table 3. Perceptions of sign language experts, mothers and caregivers on the acceptability of the nutrition cartoon video

Pre-testing aspect	*Theme*	*Sub-theme*
Acceptability	Offensive feature(s) in the video	Not properly interpreted words by the sign language interpreter
		Some disagreeable actions like throwing of plates and allowing the son to sleep with a full stomach
		Some parts might be too deep for kids
	Confusing section(s) in the video	Some sign language used are incorrect
		The transition of the video from one story to another
		The implication of breaking the *Pinggang Pinoy*® into four parts
		The connection between nightmares and eating with a full stomach
	Unbelievable section(s) in the video	The sign language interpreter's expression for "angry"
		The nightmare and being in a fictional world
	Suggestions to make the video more acceptable to watch	To review the sign languages used
		Adjustment on the size of the sign language interpreter
		Needs improvement on the transition of the story and emotions of the sign language interpreter
		Needs improvement on some colours and the voice of the boy
		Some concept in the story may not be suitable for younger children
		Add subtitles

five (5) specified illustration, and three (3) for animation. Some mentioned that the sounds and voice-over, title, the word *Pinggang Pinoy*®, characters, the use of the Filipino language, and its educational value also caught their attention.

The sign language experts said that they liked the illustration, colours, sign language interpreter, lesson of the story, voice-over, and animation. The mothers and caregivers also liked the illustration, colours, story and lesson, sounds and voice-over, characters, attractiveness, and that it suited all ages.

The four (4) experts mentioned that they disliked the sign language interpreter. The sign language interpreter did not express the emotions appropriately, signs were incomplete, and that some parts of the image in the video were covered. Additionally, the characters should have natural movements.

A total of twenty-five (25) mothers and teachers said that there was nothing in the video that they disliked. Three (3) participants said that they disliked the video because it lacked background music, some colours of the background were not suitable, and the voices of some characters were muffled.

A total of five (5) sign language experts stated that the sound level was

Table 4. Perceptions of sign language experts, mothers and caregivers on self-involvement of the nutrition cartoon video

Pre-testing aspect	*Theme*	*Sub theme*
Self-involvement	Reason/s that encourage you to follow the message in the video	The nutrition messages
		The moral lesson of the story
		Corrected sign language
		Looking forward for the sequel
		Having their own *Pinggang Pinoy*® materials
		The idea of being healthy and the importance of a proper nutrition by eating well-balanced meal
	To whom is the video message directed to?	Children
		Everyone
	Reason/s of willingness to follow the message/advice given in the video	Because it is educational and right for the kids
		Looking forward for the next episode
		Because it is for the good of their health
		Because of the moral lessons in the story
		Because of the benefits of knowing the right food proportion and well-balanced diet
		Because not only it will give them benefits as an individual, but the whole society as well

just right and only one (1) found it very soft. Among the mothers and caregivers, twenty-seven (27) found the sound level just right, and only three (3) found the sound level very loud.

A total of three (3) sign language experts considered the sign language interpreter in the video as excellent, two (2) experts said that the interpreter was good, and only one (1) said that the sign language interpreter needed improvement.

A total of twenty-two (22) mothers and caregivers stated that the video was excellent, seven (7) mothers and caregivers said that it was good, while only one (1) mentioned that it needed improvement.

All the sign language experts and eight (8) of the mothers and caregivers said that the duration of the video was just right. Only three (3) respondents stated that it was too long, while another three (3) respondents said that it was too short.

C. Perceived acceptability of the video

The video is acceptable when the message does not contain anything that is offensive or distasteful by local standards; anything that people perceive to be false; and any annoying elements that will become irritating after repeated exposure to the message.

A total of 33 of the study participants mentioned that they found the video acceptable and there was nothing in the video that they found offensive, annoying, confusing, and unbelievable. Some comments gathered from four (4) study participants included:

1. The video was offensive because of the language used, some parts of the story may be too complex for younger kids to understand, and the mother character just allowed her son to consume too much food and let him go to sleep immediately.

Table 5. Perceptions of sign language experts, mothers and caregivers on the overall impression of the nutrition cartoon video

Pre-testing aspect	*Theme*	*Sub theme*
Overall impression	Overall rating of the video	Excellent, timely, and relevant video
		Very interesting, engaging, and informative
		Suited for young viewers
		Messages that need to be conveyed are clear
		It is an educational and comprehensive video made by Filipinos
		Visuals are clear and attractive
		Needs some improvement on the sign language and sign language interpreter's facial expression
		Sound level is just right
		Needs to add background music
		Duration of the video is appropriate
		Duration of the video is ideal
		Requests to create more nutrition cartoon episodes

2. The video was annoying because there was no background music, there was too much talking without action, and the part where the prince was shouting too loud at the servants and farmers.
3. Two experts stated that they found it confusing because the words were not interpreted properly by the sign language interpreter; *Nutrilandia* was not mentioned earlier and yet the kids knew about it by the end of the video; the *Pinggang Pinoy*® was used in the video with only 1-2 pictures of representations for the Go, Grow and Glow foods; one usually get indigestion and not nightmares after eating and sleeping with a full stomach; transition of the story; and reason why the *Pinggang Pinoy*® was broken into four pieces and why the pieces were hidden in different places.
4. The video was unbelievable because the facial expression of the sign language interpreter did not change according to what was happening in the story and they found that the nightmare with the protagonist's sister and getting into another world as unbelievable.

Other comments to improve the video included: elements that needed to be changed like hand gestures of the sign language interpreter, background colours and road must be changed, some of the words being used can be changed, the garbled voice-over of the boy character, subtitles should be added, and the concept of "*nilason* or poisoning" and "*namatay* or death" may not be suitable for the character of the younger sister.

D. Perceived self-involvement of the video

Self-involvement of the video means that the message is perceived to be directed to the individuals of the targeted audience. Individuals perceive that the message is for them.

Four (4) sign language experts said that the lesson of the story and the sign language interpreter made them follow the messages that the video wanted to

convey. The mothers and caregivers stated that they will follow the messages of the video if there will be a sequel, or nutrition facts and benefits of *Pinggang Pinoy*® will be discussed. Others added that having their own *Pinggang Pinoy*® guide and the idea of being healthy will make them follow the messages in the video.

All sign language experts believed that the messages of the video were directed to children; while according to the mothers and caregivers, the video messages were directed to children, adults, teens, malnourished people, and everyone in general. All sign language experts were willing to follow the messages in the video because they reinforced the importance of eating a balanced and nutritious diet.

E. Perceived overall impression of the video

The overall impression of the sign language experts was that the messages and visuals were clear, volume was just right, the duration of the video was appropriate, and they rated the nutrition cartoon video from very good to excellent.

The mothers and caregivers mentioned that the nutrition cartoon video was excellent, very relevant, timely, and informative, but adding a lively and upbeat music could make it more interesting. They also mentioned that this was a good educational video for kids. Moreover, the video had good animation and sound effects, and the storyline would capture the interest of children.

DISCUSSION

In this study, the researchers determined the study participants' perceptions on attractiveness, comprehensibility, acceptability, and self-involvement (ACAS) of a nutrition cartoon video on healthy eating for hearing and deaf-mute children. The popularity and use of videos for classroom instruction has increased over the years. These are due to its affordability and user-friendliness of today's digital video cameras.

The study of Ramsay *et al.* (2012) suggested six primary characteristics of nutrition education video vignettes that can be used by nutrition educators in selecting and developing videos in nutrition education. These include (1) use real scenarios; (2) provide short segments; (3) present simple, single messages; (4) convey a skill-in-action; (5) develop the videos so participants can relate to the settings; and (6) support participants' ability to conceptualise the information. In the present study, four identified characteristics of nutrition education video were present in the cartoon video. The video provided short segments because it was a 6-minute video; presented simple and single message on a guide for healthy eating or *Pinggang Pinoy*® or the Go, Grow and Glow foods; conveyed a skill-in-action whereby study participants were willing to follow the messages of the video and to teach children the importance of a healthy and balanced diet at a young age. The video also supported participants' ability to conceptualise the information because as viewers, they could process the information conveyed by the video.

In this study, there were elements in the video that were found in both groups of participants like healthy eating messages, moral lessons, illustrations, and colours. Sign language construction and synchronisation of emotion while signing were elements in the comprehensibility part of the video that teachers picked up. Mothers and caregivers mentioned that some of the first things that caught their attention in the video included illustration or graphics, colours, animation, story or lesson, sounds and voice-over, title,

Pinggang Pinoy®, characters, and language used.

The findings of this study are validated by previous literature on instructional video and animation. There are some tips that should be considered in creating an instructional video as suggested by Beheshti (2018), such as clear aims of the video, simple and short, text, graphic, caption, screen recording, and animated characters. Voice is a significant factor that should be taken into account when making an instructional video as it improves engagement by guiding the viewers or learners via voice scripts (Beheshti, 2018). Another factor to be considered is animation. Animation constitutes a powerful education tool by combining audio messages with tailored visual cues and graphics. This serves the dual functions of explaining complex concepts and engaging students' interest in the learning process (Liu & Elms, 2019). A study by Kapoor (2015) mentioned that the advantage of animated character is instant engagement of learners with the learning content alongside having fun while learning. Several studies revealed that animation videos are effective in improving learning outcomes among elementary students. Animation can make students understand the topic presented more easily (Hapsari & Hanif, 2019). A video if enhanced with multimedia like pictures, animation, music and sound can motivate, attract and gather the student's attention (Yakovleva & Goltsova, 2016). This is also in line with the study of Khalid *et al.* (2010), wherein learning science was made more interesting and fun through the use of animation and cartoon.

The developed nutrition cartoon video is for introducing the concept of healthy eating through *Pinggang Pinoy*® to hearing and deaf and mute children. One of the suggestions from study participants was to include captions in the video. Caption should be considered in creating an instructional video, particularly when learners have hearing issue and are unable to watch the video with ease (Beheshti, 2018). Closed captioning is used to represent spoken and audio information as written language in real-time. It is primarily used by deaf and hard-of-hearing people (Waller & Kushalnagar, 2016). A study by Brann & Works (2011) showed that caption can enhance the video completion rate twice (40-80%). It is also useful for learners who do not have English as their first language and have issue in comprehending what is being spoken.

For the video's overall impression, sign language experts mentioned that the messages and visuals of the video were clear, volume was just right, and the duration of the video was appropriate. The length of the video is also an important factor to be considered to keep the learners or kids engaged. The 6-minute video length was in agreement with the study of Guo, Kim & Rubin (2014) that the maximum median engagement time for a video of any length is 6 minutes; thus, making videos longer than 6-9 minutes is likely to be a wasted effort. In this study, the authors observed that the median engagement time for videos less than six minutes was close to 100% for student engagement, which meant that students tended to watch the whole video.

In this study, participants also suggested to add lively music to the video to make it more interesting. This suggestion was also reported in the study of Liu & Chen (2018) that embedding appropriate background music into the video is a common way to enrich user experience, but it is a time-consuming and labour-intensive task to find music that fits the video. This was also

validated in the study by Wilver & Scalia (2017) among 215 individuals on the deaf perspective towards music. Results of this study cited the importance of music among deaf and hard-of-hearing individuals and music experience through visual or vibratory methods.

After gathering the participants' responses on the developed cartoon, their suggestions and comments were incorporated in finalising the cartoon for public consumption and roll-out to public or private schools and deaf-mute institutions in the Philippines. Results of this study will be considered in the development of other nutrition cartoon videos.

The study coverage included mothers or caregivers of hearing children for pre-testing of the nutrition video. However, the video was not pre-tested among mothers or caregivers of deaf-mute children. Children (including deaf and mute) were also not asked to evaluate the cartoon. Mothers, teachers, and children were not interviewed prior to the development of the cartoons (opinions, preference, and perspective not gathered); only a post-development assessment was made. These are the limitations of the study.

CONCLUSION

Overall, the participants held a generally positive acceptance of the video. Pre-testing the nutrition cartoon video before final production identified terminologies and concepts that the participants found unfamiliar, confusing, and unacceptable; offered suggestions for improvement that would make the pre-tested video more likely to be appropriate for hearing and deaf-mute children. Some key improvements in the video were as follows: synchronisation of the emotional expressions of the sign language interpreter like facial movements and hand gestures with the animation of video; addition of lively music or background music to make it more interesting; and inclusion of subtitles or captions in the video. Other positive findings included: the nutrition cartoon video conveyed clear information on *Pinggang Pinoy*® or healthy eating; and the messages of the video were directed to children and even for teens, adults, malnourished people, and everyone in general. It is recommended that the pre-tested nutrition cartoon video be used in nutrition education classes among hearing and deaf and mute children, and to study children's nutrition knowledge on healthy eating after watching the video. Moreover, other future studies may explore to pre-test the video among mothers or caregivers of deaf-mute children.

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Authors' contributions

IGG, principal investigator, conceptualised and designed the study, prepared the online pre-testing questionnaire, reviewed the results of pre-test, prepared the drafts and final manuscript before submission; SFQA, co-investigator, assisted in conceptualising and designing the study, prepared the online questionnaire in Google form, prepared the results of pre-test, assisted in the preparation of draft and final manuscript, assisted in reviewing the final manuscript before submission; MBSD, co-investigator, assisted in conceptualising and designing the study, assisted in the preparation of draft manuscript; TKBJ, co-investigator, assisted in conceptualising and designing the study, assisted in the preparation of results of pre-test; assisted in the preparation of draft and final manuscript; MSG, reviewed the draft manuscript, edited the draft and final manuscript.

Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Adherence to age-appropriate feeding practices among Filipino children under two: An analysis of the 2018-2019 Expanded National Nutrition Survey

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Abstract

Introduction: Age-appropriate feeding in the first two years of life is essential to a child's growth and development, with health implications that persist throughout life. This study examined the adherence to age-appropriate feeding practices among children 0-23.9 months old based on a constructed index, its association with anthropometric growth, and factors affecting adherence. **Methods:** A total of 9,138 children aged 0-23.9 months with complete information on infant and young child feeding practices (IYCF), anthropometric measurements, maternal information, and household characteristics were included in the study. Data were extracted from a nationwide survey conducted in 2018-2019. **Results:** Among children aged 0-23.9 months, 20.7% adhered to age-appropriate feeding criteria. More than half (57.7%) of children below six months adhered to age-appropriate exclusive breastfeeding practices, while 1.4% of children aged 6-8.9 months old started complementary feeding. Continued breastfeeding at 12 months of age while receiving complementary feeding was uncommon (11%). Significantly higher z-scores for weight-for-age (-0.6±0.04), height-for-age (-0.8±0.05), and weight-for-height (-0.2±0.05) were noted among children who adhered to age-appropriate feeding practices compared to those who did not. Child's age and birth weight, as well as mother's age, education, and working status, were found to significantly influence the likelihood of adhering to age-appropriate feeding index. **Conclusion:** Filipino infants and young children under two years of age have low adherence to age-appropriate feeding practices, putting them at a higher risk of repeated occurrence of infection that could lead to stunting in early life.

Keywords: childhood growth, feeding practices, nutritional status, stunting, wasting

INTRODUCTION

To ensure optimum health, growth and development, and ultimately make headway towards ending all forms of malnutrition among Filipino children under five as part of the 2030 Sustainable Development Goals (SDGs), breastfeeding and complementary feeding practices during the first two years of life need to be improved. Accordingly, the Department of Health (DOH) on May 23, 2005 issued the Administrative Order 2005-0014, titled "National Policies on Infant and Young

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Child (IYCF)", to guide efforts by the department and its partners in creating a supportive environment for appropriate IYCF practices (DOH, 2005). Since 2005, the promotion of IYCF has been one of the major nutrition programmes of the DOH and other government agencies, including local government units (LGUs), to address childhood undernutrition. Additionally, IYCF in the context of the first 1,000 days period has served as one of the strategic thrusts of the Philippine Plan of Action for Nutrition 2017-2022 to improve the nutritional status of infants and young children (NNC, 2017). Over the years, the National IYCF Plan of Action has been improved to adapt to current global strategies. This was marked by a more rigorous policy development, which included the revision of the implementing rules and regulations of Executive Order 51 (Milk Code), promotion of the Essential Newborn Care Program (known locally as *Unang Yakap*), advocacy on the availability of mother-baby friendly workplaces and public places, and the launch of the Accelerated Hunger Mitigation Program with intensive IYCF (DOH, 2005).

Breastfeeding has been widely promoted in the Philippines, with increasing rates of breastfeeding initiation and exclusive breastfeeding in the period of 2015-2018 (DOST-FNRI, 2020). However, exclusive breastfeeding practices were not sustained, as evidenced by the continuing decrease in the rate of exclusive breastfeeding until six months of age, from 77.6% for newborn infants to only 35.1% among infants under six months. This indicates that exclusive breastfeeding practice was shorter than the recommended six-month duration. Meanwhile, the prevalence of prelacteal feeding among newborns was 16.2% in 2018 (DOST-FNRI, 2020). Prelacteal feeding delays the child's first consumption of breast milk, depriving the infant of the many benefits of colostrum and breastfeeding (Som *et al.*, 2018). The IYCF programme was also challenged by the steady increase in the proportion of bottle-fed Filipino children under two years, from 44.7% in 2011 to 53.1% in 2018, with the highest rate (61.6%) noted among young children 12-15 months old (DOST-FNRI, 2020).

Despite the positive trajectories recorded in the rates of early breastfeeding initiation and exclusive breastfeeding in the Philippines, poor complementary feeding practices among young children 6-23.9 months old remains a significant challenge. A declining trend was seen in the timely introduction of complementary foods among infants aged 6-8 months, from 80.3% in 2015 to 78.8% in 2018 (DOST-FNRI, 2020). A sharp decline was also noted in the proportion of children 6-23 months old meeting the minimum acceptable diet (MAD), from 18.6% in 2015 to 13.4% in 2018 (DOST-FNRI, 2020). Poor complementary feeding during 6-23.9 months of age can directly affect children's micronutrient status and growth, given their high nutrient requirements (Dewey & Adu-Afarwuah, 2008).

Owing to sub-optimal practices on exclusive breastfeeding and complementary feeding, a marked increase in stunting during the first two years of life was reflected in recent Philippine survey estimates (DOST-FNRI, 2020). Thus, interventions focusing on preventing malnutrition, such as scaling up of nutrition-specific interventions to improve micronutrient intake of infants and young children, could help lower the high stunting and wasting prevalences in the country. This study examined the adherence to age-appropriate feeding practices among children 0-23.9 months of age based on a constructed index and its association with anthropometric growth. It also

assessed factors affecting adherence to these practices. Study findings may guide IYCF programme planners and implementers in revisiting and/or developing a comprehensive national infant and young child feeding strategy to address micronutrient deficiency and growth faltering.

METHODS AND MATERIALS

Study design, sampling, and participants

This study analysed the 2018 and 2019 data obtained from the Expanded National Nutrition Survey (ENNS) conducted by the DOST-FNRI. The ENNS 2018-2019 was a household-based rolling survey that utilised the 2013 Master Sample (MS) of the Philippine Statistics Authority as its sampling design. It aimed to generate yearly nutrition and health data for LGUs, particularly in provinces and highly urbanised cities (HUCs), for a span of three years. It employed a two-stage cluster sampling design that involved the following stages: first, the selection of enumeration areas, which were barangays or a group of adjacent small barangays, as primary sampling units; second, the selection of secondary sampling units composed of housing units/households, with at least 12-16 households in a barangay. The MS had 117 sampling domains (81 provinces, 33 HUCs, and three other areas).

Replicated sampling was employed in selecting the provinces and HUCs to provide an unbiased national estimate. The ENNS 2018-2019 covered 40 and 39 provinces and HUCs, respectively. A total of 9,138 (ENNS 2018: 4,730; ENNS 2019: 4,408) children aged 0-23.9 months with complete information on IYCF, anthropometric measurements, and other relevant data were included in this study. Further details on the survey and sampling design are published elsewhere (DOST-FNRI, 2020).

Variables measured

Anthropometric measurements were conducted using standard procedures. Weight was measured using a SECA (Model 874) double digital window scale (seca GmbH, Germany) with a 150–200 kilogram capacity, while recumbent length was measured using a medical plastic infantometer. For both weight and length, repeated measurements were done and the averages of the two measurements were computed and recorded. Child's weight and height/length were assessed using the 2006 WHO Child Growth Standard to classify the nutritional status of children. Stunting (low height-for-age) and wasting (low weight-for-height) were determined using the *z*-score cut-off points of <-2SD.

The criteria for age-appropriate feeding practices were classified based on the WHO-IYCF guidelines (WHO, 2021). Indicators were separated into indicators of breastfeeding (exclusively below six months of age) and indicators of complementary feeding practices (6-24 months of age) as shown in Table 1. Indicators were based on 24-hr recall information on breastfeeding practices, complementary feeding practices, and frequency and intake of other liquid or other semi-liquid foods. Exclusive breastfeeding was defined as the provision of breast milk solely among infants 0-5.9 months of age, although they may also receive oral dehydration solution, vitamins, minerals, and medicine in drops or syrup, and nothing else, not even water. Predominant breastfeeding, on the other hand, referred to feeding the child with breast milk as the main source of nourishment along with other liquid foods, such as water, fruit juices, and other liquid-based foods (WHO, 2008). Complementary feeding practices were evaluated using the indicators of minimum dietary diversity (MDD)

and minimum meal frequency (MMF) based on practices of the previous day (WHO, 2021). The MDD indicator was evaluated based on the consumption of at least five of the following eight food groups: 1) grains, roots and tubers; 2) legumes and nuts; 3) dairy products; 4) flesh foods; 5) eggs; 6) vitamin A-rich fruits and vegetables; 7) other fruits and vegetables; and 8) breast milk. Breastfed infants aged 6-8 and 9-23 months must receive solid, semi-solid, or soft foods at least two and three times, respectively, in the previous day to achieve MMF. Age-appropriate feeding practices were constructed to have a binary structure, i.e., whether the child adhered to the criteria or not.

An electronic data collection system containing a questionnaire was used to gather information on household, maternal, and child characteristics. Other variables considered in the assessment of potential factors included household variables, such as place of residence (rural or urban), wealth quintile (poorest, poor, middle, rich, and richest), food security status (food insecure or food secure) based on the household food insecurity access scale (HFIAS), and water and sanitation; maternal characteristics, such as age, education, civil status, working status, and place of work; and infant characteristics, including age in months, sex, birth weight, and feeding practices. For more details regarding the questionnaires used, please see earlier articles (DOST-FNRI, 2020).

Statistical analysis

Presumed variables that may influence child feeding practices, including child (age, sex, feeding practices, anthropometric growth, and birth weight), maternal (age, education, and work status), and household characteristics (type of residence, wealth status, food security status, water and sanitation, and hygiene indicators) were included in the analyses. Descriptive statistics included estimation of proportions and weighted means. Bivariate analysis was conducted and chi-square test was used to test the significance of the association between the age-appropriate feeding practices index and selected independent variables. Mean z-scores and simple linear regression analyses were done between anthropometric growth indicators and age-appropriate feeding practices index. Significant predictors of adherence to age-appropriate feeding practices, which were grouped into child, maternal, and household characteristics, were determined using univariate and multivariate logistic regression analyses. All variables with significant associations with child, maternal, and household characteristics were entered into the multivariate logistic regression analysis. The level of significance was set at $p<0.05$ for all tests performed. Analyses were performed using the Stata

Table 1. Indicators used to construct the age-appropriate feeding practices† criteria based on breastfeeding and complementary feeding practices

Age Group	*Breastfeeding*	*Complementary feeding*	
		Frequency	*Diversity*
0-5.9 months	Exclusive breastfeeding	No complementary feeding	
6-8.9 months	Breastfeeding	At least 2 feedings per day	Consumed at least 5 of the 8 food groups (MDD≥5)
9-11.9 months	Breastfeeding	At least 3 feedings per day	
12-23.9 months	Breastfeeding	At least 3 feedings per day	

MDD, minimum dietary diversity
†Based on 2021 WHO-IYCF Guidelines

version 15 (Corp LLC, Texas, USA 2017) statistical software package.

Ethical consideration

The ENNS 2018 and 2019 were approved by the FNRI Institutional Ethics Review Committee on July 31, 2017, with protocol code FIERC-2017-017. Information regarding the survey's purpose, objectives, and procedures were discussed with respondents before obtaining their oral and written informed consent to participate in the survey.

RESULTS

The ratio of boys to girls in the 2018 and 2019 surveys were both close to one (Table 1). Mean age of the children was 12.0 months, with the highest proportion being older children 12-23.9 months of age. Majority (87.3%) of the children had normal birth weight (≥2500 g).

The current feeding practices of infants under six months, six to 11 months, and young children 12 to 23 months are shown in Table 2. Exclusive breastfeeding was observed among more than half (57.7%) of infants under six months, while predominant breastfeeding was uncommon among them at only 6.3% (Table 2). More than one-fifth (13.3%) of infants under six months were already introduced to early complementary feeding while currently breastfeeding, while 16.5% received breastmilk substitute or were on a regular diet without breastfeeding. Among the six to 11 months, one-third (33.8%) of infants received breast milk substitute along with regular food. Complementary feeding in addition to breastfeeding (0.7%) was not commonly practised among infants 6-11 months, as compared to complementary feeding in addition to breast milk and breastmilk substitute feeding (8.5%). On the other hand, eeding with breastmilk substitute in addition to breast milk (sometimes referred to as mixed feeding) was commonly observed among infants 6-11 months (49.9%) and 12-23 months (39.2%), but not among infants under six months (5.5%), as shown in Table 2.

Overall, children aged 6-23 months registered a low dietary diversity score (DDS) of 3.1; both infants 6-12 months (2.5) and 12-23 months (3.4) had DDS below the recommended five food groups (Table 2). Only 4.6% infants aged 6-11 months were receiving the daily MDD of five of the eight food groups, while a higher percentage of young children 12-23 months (16.2%) met the recommended MDD. Children 6-23 months old received about six feedings per day on average, with majority of the 6-11 months (84.5%) and 12-23 months (93.7%) meeting the minimum number of feedings per day. However, most infants 6-11 months (95.4%) and 12-23 months (85.1%) failed to receive the minimum acceptable diet. Moreover, consumption of nutrient-dense foods was particularly low. Only 21.6% of infants aged 6-11 months, and 14.0% of children aged 12-23 months were fed eggs and flesh meat. Most (71.5%) children also lacked fruits and vegetables in their diet. It was also noted that consumption of sweetened beverages increased with age, from 0.9% consumption among infants 6-11 months old to 7.1% among young children 12-23 months old.

Across all ages, one-fifth (20.7%) of the children adhered to age-appropriate feeding criteria in 2018 and 2019. Meanwhile, more than half (57.7%) of the children below six months of age adhered to age-appropriate exclusive breastfeeding, while a very small proportion (1.4%) of children 6-8.9 months old started complementary feeding at an appropriate age. Continued breastfeeding at 12 months of age while receiving complementary feeding was uncommon at 11.0% (Table 2).

Table 2. Descriptive characteristics of the children, Philippines, 2018-2019

Characteristics	*2018 (n=4,730)*	*2019 (n=4,408)*	*All (n=9,138)*
Child characteristics			
Sex (%)			
Boys	51.7	50.7	51.2
Girls	48.3	49.3	48.8
Age (months)			
Mean age	12.1	11.8	12.0
0-5.9 (%)	23.9	26.0	24.9
6-8.9 (%)	12.3	13.2	12.7
9-11.9 (%)	13.6	12.4	13.0
12-23.9 (%)	50.2	48.4	49.3
Birth weight (grams) (%)			
<2500	12.9	12.5	12.7
≥2500	87.1	87.5	87.3
Current feeding practices (0-5 months) (%)			
Exclusive breastfeeding	55.4	59.9	57.7
Predominant breastfeeding	7.9	4.6	6.3
Breastfeeding + BMS[†]	3.7	7.3	5.5
Breastfeeding + CF[‡]	14.1	12.3	13.2
Breastfeeding + BMS + CF	0.8	1.0	0.9
No breastfeeding- only BMS and/or regular diet	18.1	14.9	16.5
Current feeding practices (6-11 months) (%)			
Exclusive breastfeeding	5.2	4.2	4.7
Predominant breastfeeding	3.3	1.4	2.4
Breastfeeding + BMS[†]	45.6	54.5	49.9
Breastfeeding + CF[‡]	0.7	0.7	0.7
Breastfeeding + BMS + CF	8.9	8.1	8.5
No breastfeeding- only BMS and/or regular diet	36.3	31.1	33.8
Current feeding practices (12-23 months) (%)			
Exclusive breastfeeding	0.0	0.2	0.1
Predominant breastfeeding	0.2	0.2	0.2
Breastfeeding + BMS[†]	36.3	42.4	39.2
Breastfeeding + CF[‡]	0.2	0.0	0.1
Breastfeeding + BMS + CF	8.5	7.5	8.0
No breastfeeding- only BMS and/or regular diet	54.7	49.6	52.3
Complementary feeding practices (6-23 months)			
Mean Dietary Diversity Score[§]	3.1	3.0	3.1
6-11 months	2.5	2.5	2.5
12-23 months	3.4	3.3	3.4
Met MDD (%)	13.8	10.4	12.2
6-11 months	4.5	4.6	4.6
12-23 months	18.6	13.4	16.2
Mean Meal Frequency	5.9	5.4	5.7
6-11 months	5.2	5.0	5.1
12-23 months	6.2	5.7	5.9

Table 2. Descriptive characteristics of the children, Philippines, 2018-2019 *(continued)*

Characteristics	*2018 (n=4,730)*	*2019 (n=4,408)*	*All (n=9,138)*
Met MMF (%)	89.3	91.9	90.6
6-11 months	80.6	88.7	84.5
12-23 months	93.8	93.7	93.7
Met MAD (%)	12.9	9.6	11.3
6-11 months	4.5	4.6	4.6
12-23 months	17.3	12.2	14.9
Children who consumed eggs and meat (%)	48.8	47.2	48.0
6-11 months	19.7	23.7	21.6
12-23 months	63.9	59.6	61.8
Children who consumed sweet beverages (%)	4.7	5.2	5.0
6-11 months	0.9	0.9	0.9
12-23 months	6.7	7.5	7.1
Children with no intake of fruit/vegetable (%)	70.6	72.6	71.5
6-11 months	82.3	83.7	83.0
12-23 months	64.5	66.7	65.5
Meeting age-appropriate feeding practices (%)	20.3	21.1	20.7
Age (in months, %)			
0-5.9	55.4	59.9	57.7
6-8.9	1.8	1.0	1.4
9-11.9	5.2	6.7	5.9
12-23.9	12.3	9.5	11.0
Nutritional status			
Underweight (%)	14.3	13.0	13.7
Stunted (%)	24.1	15.3	19.8
Wasted (%)	7.5	4.2	5.9
Overweight/Obese (%)	3.4	2.6	3.0
Maternal characteristics			
Age (years)			
Mean age	29.1	29.3	29.2
<20 (%)	6.3	7.1	6.7
20-29 (%)	51.2	49.8	50.5
≥30 (%)	42.5	43.2	42.8
Education (%)			
No education/informal education	0.8	2.0	1.3
Elementary	13.2	16.3	14.7
At least high school	58.3	59.6	58.9
At least college	27.7	22.2	25.0
Currently working (%)			
Working	26.7	18.9	22.9
Not working	73.3	81.1	77.1
Household characteristics			
Residence (%)			
Rural	50.5	58.0	54.1
Urban	49.5	42.0	45.9

Table 2. Descriptive characteristics of the children, Philippines, 2018-2019 *(continued)*

Characteristics	*2018 (n=4,730)*	*2019 (n=4,408)*	*All (n=9,138)*
Wealth Status (%)			
Poorest	24.1	22.1	23.1
Poor	22.4	23.7	23.1
Middle	21.2	21.9	21.6
Rich	17.7	18.8	18.2
Richest	14.5	13.5	14.0
Food security[¶] (%)			
Food secure	34.0	26.4	31.8
Food insecure	66.0	73.6	68.2
Source of drinking water (%)			
Improved	96.8	96.2	96.5
Not improved	3.2	3.8	3.5
Type of latrine (%)			
Water sealed	94.3	93.7	94.0
Not water sealed	2.8	2.7	2.7
No toilet	3.0	3.6	3.3

[†]BMS=breastmilk substitute
[‡]CF= complementary feeding
[§]Mean dietary diversity score was determined based on the eight (8) food groups in the 2021 WHO-IYCF guidelines: breastmilk, grains, roots and tubers and plantains, dairy products, legumes and nuts, flesh foods, eggs, vitamin-A rich fruits and vegetables, and other fruits and vegetables
[¶]Food security was assessed using the HFIAS

The stunting prevalence among children in 2018 and 2019 was about 20%, while 13.7% of children were underweight and 6.0% were wasted. Mothers of the children were predominantly 20 years old and above (93.3%), with a mean age of 29.2 years old; most had a minimum of high school education (58.9%) and were not working (77.1%) at the time of survey. A higher proportion of children were from rural (54.1%) than urban areas (45.9%). Households were almost equally represented across the poorest, poor, and middle-income groups, while a lower proportion of households belonged to the rich (18.2%) and richest (14.0%) income groups. The majority of households were food insecure (68.2%), although almost all households had improved water sources (96.5%) and latrine (94.0%) (Table 2).

Table 3 presents child characteristics according to the constructed age-appropriate feeding practices based on the 2021 WHO-IYCF indicators. Using the index for nutritional status, the mean weight-for-age (WAZ), height-for-age (HAZ), and weight-for-height z-scores (WHZ) were significantly higher among children who adhered to age-appropriate feeding practices (-0.6, -0.7, and -0.2, respectively) compared to those who did not adhere to the recommendations. Significantly, a higher prevalence of stunting (83.1%; $p<0.001$), underweight (84.8%; $p<0.001$), and overweight (67.2%; $p=0.002$) was recorded among children who did not adhere to age-appropriate feeding practices than those who followed the recommendations. Moreover, non-adherence to age-appropriate breastfeeding practices was significantly higher among low-

Table 3. Child characteristics by age-appropriate feeding practices index, Philippines 2018-2019

Variables	*Adherence to age-appropriate feeding criteria*		*p-value*
	No	*Yes*	
Child characteristics			
Sex (%)			0.691
Boys	79.0	21.0	
Girls	79.6	20.4	
Nutritional status			
Mean WAZ	-0.8	-0.6	<0.001
Mean HAZ	-1.1	-0.7	<0.001
Mean WHZ	-0.4	-0.2	<0.001
Underweight (%)	84.8	15.2	0.001
Stunted (%)	83.1	16.9	<0.001
Wasted (%)	79.9	20.1	0.823
Overweight/Obese (%)	67.2	32.8	0.002
Birth weight (grams) (%)			0.015
<2500	82.7	17.3	
≥2500	78.8	21.2	
Maternal characteristics			
Age (years) (%)			0.057
<20	76.5	23.5	
20-29	78.5	21.5	
>29	80.6	19.4	
Education (%)			0.001
No education/formal education	90.8	9.2	
Elementary	80.6	19.4	
At least high school	77.7	22.3	
At least college	81.9	18.1	
Others	79.8	20.2	
Currently working (%)			<0.001
Working	86.6	13.4	
Not-working	77.1	22.9	
Household characteristics			
Residence (%)			0.391
Rural	78.8	21.2	
Urban	79.8	20.2	
Wealth status (%)			0.281
Poorest	77.0	23.0	
Poor	78.8	21.2	
Middle	79.3	20.7	
Rich	81.1	18.9	
Richest	81.3	18.7	
Food security† (%)			0.411
Food secure	79.7	20.3	
Mildly FI	76.6	23.4	
Moderately FI	78.0	22.0	
Severely FI	80.0	20.0	
Source of drinking water (%)			0.138
Improved	79.4	20.6	
Not improved	76.3	23.7	
Type of latrine (%)			0.123
Water sealed	79.2	20.8	
Not water sealed	84.2	15.8	
No toilet	76.0	24.0	

WAZ, weight-for-age z-score; HAZ, height-for-age z-score; WHZ, weight-for-height z-score; FI, food insecure
†Food security was assessed using the HFIAS.

Table 4. Association between adherence to age-appropriate feeding practice and mean anthropometric z-scores of children 0-23 months old, Philippines 2018-2019

Status of adherence to age-appropriate feeding practices by z-score	*Mean z-score*				*Bivariate linear regression*			
	Mean	*SE*	*95%CI*		*Coeff*	*p-value*	*95%CI*	
			LL	*UL*			*LL*	*UL*
WAZ								
No	-0.84	0.03	-0.89	-0.78	reference			
Yes	-0.59	0.04	-0.68	-0.50	0.25***	<0.001	0.15	0.34
HAZ								
No	-1.08	0.04	-1.17	-1.00	reference			
Yes	-0.75	0.05	-0.85	-0.64	0.34***	<0.001	0.25	0.42
WHZ								
No	-0.36	0.02	-0.40	-0.31	reference			
Yes	-0.17	0.05	-0.27	-0.07	0.18**	0.004	0.07	0.30

WAZ, weight-for-age z-score; HAZ, height-for-age z-score; WHZ, weight-for-height z-score
*p<0.05, **p<0.01, ***p<0.001

birth weight infants (82.7%; p=0.015) than among infants with normal birth weight. On the other hand, a higher percentage of adherence was noted among children whose mothers were less than 20 years old, although this was statistically insignificant. Children born from mothers with no formal schooling and were working (9.2%, and 22.9%, respectively; p<0.001) were less likely to adhere to age-appropriate feeding practices. No significant difference was noted between sex and across socio-economic and demographics characteristics in terms of adherence to age-appropriate feeding practices (Table 3).

Table 4 shows the association between adherence to age-appropriate feeding practices and mean z-scores of children 0-23 months old. Children who followed age-appropriate feeding practices had significantly higher mean WAZ at -0.59 (95% CI: -0.68; -0.50), mean HAZ at -0.75 (95% CI: -0.85; -0.65), and mean WHZ at -0.17 (95% CI:-0.27; -0.07) compared to children who failed to meet feeding guidelines. Correspondingly, adherence to age-appropriate feeding practices had a positive effect on mean WAZ (β=0.25, p<0.001), HAZ (β=0.34, p<0.001), and WHZ (β=0.18, p=0.004) of children under two years based on the results of the bivariate analysis.

Regression analysis revealed the predictors of adherence to age-appropriate feeding practices among children 0-23 months old, as shown in Table 5. The likelihood of meeting age-appropriate feeding practices increased as the child grows older, from 6-8.9 months to 12-23.0 months, at OR=0.01 (95% CI=0.01-0.02) to OR=0.09 (95% CI=0.07-0.11), respectively (Table 5). Children with normal birth weight (OR=1.44; 95% CI=1.11-1.86) were more likely to meet age-appropriate feeding practices compared to children with low birth weight. Children born from mothers with an educational attainment of high school level or better (OR=2.45; 95 CI%=1.01-5.65) were more likely to meet age-appropriate feeding practices than their counterparts. Children whose mothers were not working (OR=1.97; 95 CI%=1.53-2.54) were also more likely to exhibit adherence compared to children whose mothers were working at the time of survey.

Table 5. Multivariate regression analysis for predictors of adherence to age-appropriate feeding practices among children 0-23 months old, Philippines, 2018-2019

Variables	*Bivariate logistic regression*				*Multivariate logistic regression*			
	Crude OR	*p-value*	*95%CI*		*Adjusted OR*	*p-value*	*95%CI*	
			LL	*UL*			*LL*	*UL*
Age (months)								
0-5.9	reference				reference			
6-8.9	0.01***	<0.001	0.01	0.02	0.01***	<0.001	0.01	0.02
9-11.9	0.05***	<0.001	0.03	0.06	0.04***	<0.001	0.03	0.06
12-23.9	0.09***	<0.001	0.07	0.11	0.09***	<0.001	0.07	0.12
Birth weight (grams)								
<2500	reference				reference			
>=2500	1.29*	0.016	1.06	1.57	1.44**	0.009	1.11	1.86
Maternal characteristics								
Education								
No education	reference				reference			
Elementary	2.37	0.084	0.88	6.41	2.09	0.116	0.81	5.38
At least high school	2.83*	0.031	1.11	7.21	2.39*	0.047	1.01	5.65
At least college	2.18	0.108	0.82	5.74	1.96	0.111	0.84	4.55
Others	2.50	0.121	0.76	8.20	2.47	0.173	0.64	9.46
Currently working (%)								
Working	reference				reference			
Not working	1.92***	<0.001	1.53	2.40	1.97***	<0.001	1.53	2.54

*p<0.05, **p<0.01, ***p<0.001

DISCUSSION

Malnutrition, particularly stunting, underweight, and wasting, was identified as a significant public health concern among young Filipino children under two in the Philippines in 2018-2019. Employing a constructed age-appropriate feeding practices index, this study assessed the impacts of age-appropriate feeding on children at the age recommended for exclusive breastfeeding through the complementary feeding period to a family diet, while also exploring their association with malnutrition prevalence. This represents an initial attempt to consider child feeding practices in terms of appropriateness for age, including the quality and quantity of complementary meals, as part of the criteria set in the new IYCF guidelines released in 2021 (WHO, 2021).

Study results indicated that more than half of the children below six months of age adhered to age-appropriate exclusive breastfeeding practices, while only a very small proportion of children (1.4%) started complementary feeding following age-appropriate criteria (i.e., breastfeeding with at least two feedings per day and consumption of at least five of eight food groups). Also, continued breastfeeding at 12 months of age while receiving complementary feeding was not common among Filipino children. Thus,

it is important to refocus and reinforce the message to health practitioners to promote appropriate breastfeeding practices in the Philippines. The first two years of an infant's life is the most rapid growth period when breast milk is no longer enough to meet the nutritional requirements of infants; they need to be fed with various types of complementary foods in addition to breast milk. Thus, poor diets and feeding practices play a major role. In fact, research has shown that children in South Asia were more likely to become stunted and wasted if they began their complementary foods too late, consumed too few meals, and lacked dietary diversity (Torlesse & Aguayo, 2018). The findings of a meta-analysis conducted in Bangladesh (Islam *et al.*, 2020) reported that initiation of complementary feeding at or after seven months of age increased the risk of stunting by 1.23 times. This is also consistent with findings in the Philippines where untimely introduction of complementary foods (either too early or too late) increased the child's odds of being stunted by almost twice and of being severely stunted by more than four times (Guirindola, *et al.*, 2021).

Unfortunately, there was a high prevalence of bottle feeding among infants 6-11 months and 12-23 months, ranging from 33.8% to 52.3%, respectively; while less than 60% were exclusively breastfed during the first 6 months. The proportion of infants 6-11 months old receiving complementary feeding along with breast milk was low, as most of them were given a mixed feeding of breast milk and breastmilk substitute, including feeding solely on breastmilk substitute. It is therefore essential to promote breastfeeding practices in the Philippines. The weak enforcement of the Executive Order 51 or the Philippine Milk Code on Marketing of Products related to Infant and Young Child Feeding, and the growing advertising and physician's recommendations of breastmilk substitute products, were identified as major factors that influenced mother's decision to feed infant formula (Sobel *et al.*, 2011). Moreover, ingestion of unsafe drinking water with bacterial contamination is one of the major causes of diarrhoea (Rodriguez *et al.,* 2011) and this increases the risk of stunting and malnutrition. Bottle feeding with infant formula using unsafe water is therefore an important concern in preventing the risk of undernutrition among infants and young children in the Philippines.

Furthermore, results of the study also underscored the decreasing adherence to the criteria for appropriate daily feeding practices during the transition period from breastfeeding to a regular diet. Adherence to age-appropriate feeding practices was especially affected by the quality of complementary foods, emphasising the lack of diversity in children's diet as shown by a mean DDS of only 3.1, which was below the recommended consumption of at least five of eight food groups. In addition to lack of dietary diversity, more than 80% of the infants and young children did not receive MAD, highlighting that the quality of their diet is still a major concern in the Philippines. Consumption of nutrient-dense foods, such as meat, poultry, fish, egg, and fruits and vegetables, was also low among infants. Indeed, the lack of diversity in complementary foods given to infants and young children, rather than the number of meals consumed, was the main bottleneck of not meeting the international recommendation for MAD.

The limited capacity to provide quality complementary foods to young children as measured by MAD can have irreversible impacts on the mental and physical growth of children, which can lead to poor cognitive development and

limited opportunities for work throughout life. This corroborates with the finding of this study where adherence to the recommended age-appropriate feeding practices was positively associated with child growth. In particular, Filipino children who adhered to the recommended practices had significantly higher mean z-scores (WAZ, HAZ, and WHZ) as opposed to those who did not. Correspondingly, a higher prevalence of stunting, underweight, and overweight was noted among children who failed to adhere to age-appropriate feeding practices relative to those who observed the recommended practices. Several studies have documented the positive association between dietary diversity indicator and child anthropometry (Jones *et al.*, 2014; Mallard *et al.*, 2014; Ruel, Harris & Cunningham, 2013), particularly on linear growth (HAZ), and in some cases, for WHZ and WAZ (Mallard *et al.,* 2014; Ruel *et al.*, 2013). In addition, a pooled analysis of 14 demographic and health survey data sets (2003–2006) from Africa and South Asia showed that among infants 6–8 months of age, consumption of solid foods was associated with a lower risk of both stunting and underweight (Marriot *et al.*, 2012). In their model for children 6–23 months of age, Marriot *et al.* (2012) found that meeting MDD, consuming iron-rich foods, and achieving MAD were also associated with a lower risk of both stunting and underweight. Meanwhile, consuming the recommended minimum number of meals was associated only with underweight prevalence.

A final aim of the study was to identify factors affecting adherence to age-appropriate feeding practices in children under two. The positive association between a child's age and adherence to age-appropriate feeding showed the poorer quality of feeding practices among youngest infants (0-5.9 months), who were supposed to be receiving exclusive breastfeeding, compared to older infants six months and above. This could be explained in the context of complementary feeding; wherein older children were more likely to increase their intake of semi-solid and solid foods than younger children 6-8.9 months old. This is not surprising since semi-solid and solid foods are usually introduced gradually and stomach capacity at six months is relatively limited. This result is consistent with studies in Benin Africa (Mitchodigni *et al.,* 2017), West Africa (Isaaka *et al.,* 2014), and Ethiopia (Melkam *et al.*, 2013). In addition, children with perceived normal birth weight were more likely to adhere to appropriate feeding practices. Specifically, a higher likelihood of meeting DDS, MMF, and MAD was related to children having average or above-average birth weight. This could be influenced by the belief that children with below-average birth size may have lesser capacity for digestion.

The mother's education level was identified as a very important predictor of adherence, suggesting that mother's literacy and household living standards are the main drivers of a child's adherence to appropriate feeding practices. A study in Cambodia (Hondru *et al.,* 2020) noted that while household living standards could be improved, higher educational attainment among mothers was associated with better feeding practices and growth outcomes, owing to educated mothers having better access to information, services, and materials promoting appropriate feeding practices. The same trend has been reported in Poland (Kostecka, Jackowska & Kostecka, 2020) where older and better educated mothers were more likely to exclusively breastfeed their children for a longer period than younger mothers. A significant association

was also found between the education status of mothers and appropriate complementary feeding practices in a study conducted in Ethiopia (Demilew, Tafere & Abitew, 2017; Mekonnen *et al.,* 2021), which the authors assumed was attributable to better comprehension of nutrition education among educated mothers as opposed to those who had less educational opportunities or had no formal schooling. The authors also highlighted that educated mothers were more likely to read books, flyers, and other forms of educational materials about IYCF. Evidence from literature and the results of this study support the need for interventions among mothers to improve feeding practices with a holistic view on health and nutrition to address malnutrition among young children.

Similar to the Bangladesh study (AffifaTanny *et al.,* 2019), our results revealed that non-working mothers or housewives were more likely to adhere to appropriate feeding practices than working mothers. For instance, although exclusive breastfeeding was more prevalent among the group of working mothers with infants up to 3 months old compared to the group of housewives; housewives with infants up to six months old, however, practised exclusive breastfeeding more than working mothers. This was attributed to working mothers' inability to sustain exclusive breastfeeding due to insufficient knowledge regarding expressed breast milk feeding and unfavourable working environment that discourages breastfeeding. Indeed, developing better awareness on the importance of exclusive breastfeeding practices, nutritious and diverse complementary foods, and putting efforts in communicating the age-appropriate feeding practices to meet their nutritional needs are key.

Strengths and limitations of the study

The main strength of this study relates to the large number of study participants covered in the ENNS 2018 and 2019, which described population trends and produced valuable evidence. Data collection methods, including anthropometric and dietary assessments from which IYCF indicators were derived, followed international standard protocols to ensure measurement accuracy.

Several limitations were noted in this study. Dietary intake estimates using the 24-hour recall for infants and young children were based on reports by parents and may have included inaccuracies, leading to over- or under-estimation of food intakes. Another limitation of the 24-hour food recall is the possibility that the entire dimension of feeding practices, as well as the presence of other risk factors, cannot be fully captured. Additionally, the risk of misclassification and social desirability biases during interviews must be considered.

CONCLUSION

High prevalences of stunting, underweight, and wasting were revealed as significant public health concerns among young Filipino children under two in 2018 and 2019. Meanwhile, low adherence to age-appropriate feeding practices based on feeding guidelines remains a major concern among children 0-23 months of age. Children's adherence to age-appropriate feeding practices index was significantly associated with anthropometric growth, wherein mean z-scores (WAZ, HAZ, and WHZ) were found to be higher among children who adhered to age-appropriate feeding practices than those who did not. Indicators, such as child's age and birth weight, as well as the mother's

educational attainment and working status, were found to significantly influence the likelihood of adherence.

These findings can provide guidance to IYCF programme planners and implementers in revisiting and developing a comprehensive national infant and young child feeding strategic plan to address micronutrient deficiency and growth faltering during the first 1,000 days. Firstly, awareness campaigns on the importance of adherence to age-appropriate breastfeeding and complementary feeding should be intensified as a potential intervention to reduce the risk of undernutrition. Secondly, activities to improve education level, as well as the health knowledge and practices of women across the country is crucial. Lastly, optimal breastfeeding practices should be promoted while the quality of complementary feeding practices should be enhanced by increasing diversity, meal frequency, and overall acceptability to ensure optimum child growth and development among Filipino infants and young children 0-23 months of age.

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Authors' contributions

EAG, conceived, carried out the study, reviewed and edited the manuscript; MLVM, analysed and interpreted the data. All authors read and approved the manuscript.

Competing interest

The authors declare that they have no competing interests.

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Dominant factors on food coping mechanism of poor households in Pringsewu Regency, Indonesia

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ABSTRACT

Introduction: In general, poor households do not have sufficient purchasing power to ensure food security, and this has led to the establishment of food coping mechanisms to alleviate this insufficiency. Therefore, this study was aimed at identifying the dominant factors on food coping mechanism of poor households. **Methods**: Simple random sampling technique and random tables were used to obtain the data from a sample size of 92 beneficiaries of poor targeted households of prosperous rice (RASTRA) in Pringsewu Regency through interviews and a questionnaire. Four villages, i.e., Fajar Baru, Kemilin of North Pagelaran Sub-District, Wargomulyo, and Tanjung Rusia of Pardasuka Sub-District, were the locations of the study. Data were analysed using descriptive and factor analysis. **Results:** Results showed that most households had food coping mechanisms involving buying smaller amounts and cheaper types of foods. Factors forming the poor households' food coping mechanisms were the households' social and economic conditions, food coping activities, assets, and heads' and their wives' occupations. **Conclusion**: In Pringsewu Regency, the dominant factor affecting food coping mechanism was social condition. This condition encompassed the household head's age, his and his wife's length of formal education, their nutrition knowledge, and the number of actors on food coping mechanism. Education was the dominant variable on food coping mechanism of poor households and it played the biggest role in affecting the establishment of survival mechanisms for overcoming food insecurity.

Keywords: dominant factor, food coping, RASTRA

INTRODUCTION

Poverty and starvation are some of the most basic humanitarian problems. In 2015, the Food and Agriculture Organization (FAO) reported that starvation affected 795 million people worldwide (720-811 million people in the world faced starvation in 2020); 780 million of whom lived in developing countries, including Indonesia (FAO, IFAD & WFP 2015; Glazebrook, Noll & Opoku, 2020). This led to the Sustainable Development Goals (SDGs), intended to overcome starvation. SDGs is a sustainable development agenda agreed by various countries of the United

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Nations (UN) based on human rights and equality for the promotion of social, economic, and environmental aspects.

The global agenda proposed by SDGs for overcoming poverty and starvation is similar to the national priority of human growth in the Medium-Term Development Plan 2015-2019 (RPJMN) and Nawacita Programme (President's programme) in Indonesia. According to the Food Act Number 12, 2012, the state is mandated to provide the population's food requirements under Human Rights (HAM). As a basic necessity and strategic commodity, food plays an essential role in making sure humans survive with healthy and productive lives.

One of the Indonesian government's efforts to tackle starvation and food insecurity issues is the food security programme. According to the decree of the Ministry of Agriculture No. 14/ Permentan/Ot.140/3/2012, the food security programme is regarded as a national development priority. The ministry developed the BULOG (Logistic Affairs Agency) as a State-Owned Enterprise (BUMN) tasked with supporting the food affordability sub-system through the supply and distribution of subsidised rice to the poor. It is known as the Prosperous Rice Programme (RASTRA), a food subsidy programme aiming to minimise the expenditure of the target households by fulfilling some of their basic food needs (rice) and preventing any decrease in energy and protein consumption. This has positively contributed to the people of the class by opening economic and physical accesses to foods, preventing malnutrition and providing energy, as well as protein (BULOG, 2014). The provision of RASTRA is based on the fact that food expenditure is dominated by rice, hence the poor are very vulnerable to the inability to afford rice as a result of the rise in its prices. The Indonesian government therefore seeks to provide sufficient food for poor households through RASTRA by distributing 15 kilograms to each household monthly.

Food insufficiency is a threat to the households' food security and nutritional status. One of the major factors of food security is household income. Household income is related to a household's poverty level (Grobler & Dunga, 2017), which can result in low purchasing power (Yousaf *et al.,* 2018). Therefore, the poor make various efforts to provide sufficient food for their household members. Besides RASTRA, households also carry out food coping mechanisms to handle food shortages and economic limitations (Anggrayni, Andrias & Adriani, 2015). This process is performed when the difficulty in fulfilling food needs emerges or due to the lack of capability to meet consumption needs for all members (Ume, Ci & Gbughemobi, 2018). Food insecurity is a factor driving households to take on food coping mechanisms.

According to Abdulla (2015), every household's food coping mechanism depends on the situation of food shortage and their ability to deal with it. Negash *et al.* (2015) stated that households with unemployment and unstable income problems have better coping mechanisms. The higher the food insecurity rate, the greater the coping mechanism taken on by the household (Grobler & Dunga 2017). Furthermore, regions with better food security have adaptive coping mechanisms (Ghimire, 2014). Therefore, food-insecure households' characteristics play a vital role in their coping mechanism (household socio-demography) (Alam, 2017).

One of the Food Development Goals is the realisation of food security at the household level and Lampung Province is one of the areas classified as a food-secured region. Regionally, Pringsewu Regency is one of the regions of Lampung Province with a surplus of rice, but has

malnutrition cases involving children under five years old. In the availability aspect, Pringsewu Regency has a surplus of 48,116 tons of rice and also corn and cassavas as sources of carbohydrates (Badan Ketahanan Pangan (BKP) Pringsewu or Food Security Agency, 2015[a]). However, regional food security does not guarantee household and individual food security (Suhaimi, 2019). There are still high rates of food insecurity among households, which indirectly affects children's overall nutritional status. Based on published data, the number of under-five-year-old children suffering from malnutrition in Pringsewu Regency during 2012-2015 was 26. This number increased by 80 percent from 2014 to 2015 (BKP Pringsewu, 2015[b]). Malnutrition is closely related to poverty. Pringsewu Regency's poverty line is at IDR 408.174/capita/month or 11.50% of the population in 2018 (BPS Pringsewu, 2021). The number of poor and food-insecure citizens in Pringsewu Regency was 45,580 in 2015. This number increased by 20.7 percent from 2014 to 2015 (BAPPEDA Pringsewu, 2016). This shows that Pringsewu Regency still faces chronic food insecurity due to poverty caused by unequal income distribution and monthly expenditure per capita as a result of the population's inability to handle food expenditure. Despite having a surplus, the presence of cases associated with malnutrition still indicates food security problems. One crucial problem is low access to foods, which led to the establishment of the RASTRA programme.

This study identified the food coping mechanisms of RASTRA-recipient households and determined how they were carried out. It also provided recommendations regarding developing and formulating appropriate policies to solve food insecurity issues through the understanding of the strategies adopted by these poor households.

MATERIAL AND METHODS

Locations and research time

This study was conducted in Pringsewu Regency using the survey method, and the location was determined purposively. There are nine districts in Pringsewu Regency. Pardasuka and North Pagelaran Sub-District were selected as the study locations based on poverty and composite indicators on the Food Security and Vulnerability Atlas (FSVA) [BKP Pringsewu, 2015[b]]. These indicators were used to choose locations with populations below the poverty line as the first priority. In terms of composite index, food insecurity in an area is caused by indicators such as availability, access, and nutrition utilisation. The availability indicator is based on the normative consumption ratio of net availability per capita per day. The access indicator is based on the poverty line, proportion of expenditure, and access to electricity. The nutrition utilisation indicator is based on the length of schooling for girls, access to clean water, ratio of the population per health workers to level population density, prevalence of stunting for toddlers, and life expectancy at birth (BKP of Ministry of Agricuture, 2018). The selected areas were characterised by the food insecurity status and a composite index value of 3 in December 2015. This composite index value indicated that Pardasuka and North Pagelaran Sub-districts fell into the category of first-degree food insecurity. Four villages, namely Fajar Baru, Kemilin in North Pagelaran Sub-District, Tanjung Rusia, and Wargomulyo in Pardasuka Sub-district, were chosen as the study locations as they had the largest numbers of RASTRA beneficiaries. The data were collected from July to August 2018.

Sampling technique

The population was 1,132 poor

households receiving RASTRA in 2015. However, the number of households as valid sample units determined based on Slovin's formula with an estimated error rate of 10 percent was 92 (Siregar, 2016). The number of sample units involved in this study was estimated using the formula below:

$$n = \frac{N}{1 + N.e^2}$$

n : the number of sample units
N : total population (1,132 poor households)
e : margin of error (0.1 atau 10%)

The minimum acceptable sample size based on the correlational descriptive methods using statistical data analysis was 30 (Louangrath, 2017). So, the number of sample units in this study was acceptable. In addition, the number of sample units was considered large enough for factor analysis and validated reliability. For each village, the number was determined proportionally, and the number of sample units in Fajar Baru Village, Kemilin, Tanjung Rusia, and Wargomulyo were 13, 19, 26, and 34 households, respectively. The different households were selected using simple random sampling technique by random-table.

Data collection and data analysis techniques

Primary and secondary data were employed in this study. The primary data were obtained through direct observation and interviews using a structured questionnaire adapted from Usfar (2002). The items of the questionnaire included the characteristics of poor households (age, gender, education, number of family members, nutrition knowledge, job, income), food expenditure, assets, amount of rice availability, and food coping strategy activities (types of activities, frequencies, and actors). The secondary data were collected from agencies or institutions related to the study, such as data on the number of poor households receiving RASTRA, Food Security Vulnerability Atlas (FSVA), undernutrition, food availability, and expenditure of the population in Pringsewu Regency. Then, the descriptive-analytical method was applied to determine the households' food coping mechanisms based on the types of activities, frequencies, and actors (Negash *et al.,* 2015).

The food coping mechanisms conducted by each household depended on the food problems faced. All types of food coping activities indicated the existence of household food insecurity problems, but they did not necessarily determine the same level of severity. Therefore, the greater the food insecurity, the more significant the mechanism. Behaviours were grouped into three categories and assigned a scale value. Scale 1 included actions to increase income, change eating habits, and increase immediate access to foods. Scale 2 included measures to increase immediate access to foods, change distribution and frequency, and go through days without eating. In contrast, scale 3 was a drastic step, such as migration, giving children to relatives, and divorcing. The larger the scale, the more severe the food problems faced that increased the use of food coping mechanisms. The severity of food coping mechanisms can also be affected by the socio-demographic characteristics of the households.

This study applied quantitative descriptive analysis for the food coping mechanisms undertaken by poor households and factor analysis composing of the Principal Component Analysis (PCA) extraction model to figure out the dominant factors of food coping mechanisms based on the socio-demographic characteristics

of households. Household socio-demographic variables with a strong correlation were included in the factor analysis and variables with a weak correlation were excluded from the factor analysis. If one or several initial variables individually had a Measure of Sampling Adequacy (MSA) value of lower than 0.5, then the variable was removed from the analysis process; so, it was necessary to repeat the analysis until all variables had a MSA value of higher than 0.5. The next step was to test the adequacy of the sample through the Kaiser-Meyer-Olkin (KMO) Measure of Sampling Adequacy. If the KMO value was between 0.5 and 1, it was concluded that the appropriate factor analysis had been used. Bartlett's Test of Sphericity was used to determine the significant correlations between variables. Then, the eigen value showed the total variance that could be explained by each factor. Extracting the factors required looking at an eigen value greater than or equal to 1.0 (Santosa, 2012).

Ethical approval

This study was conducted under the approval of the Ethics Committee of the Institute of Research and Community Service, University of Lampung. All participants were informed of the purpose of the study and gave their written consent for voluntary participation.

RESULTS AND DISCUSSION

Characteristics of poor households

The food coping mechanism behaviour performed by each household was different and depended on their finance. Besides, socio-economic factors also affected various alternatives chosen by the households as survival mechanisms, and these characteristics significantly affected their access to food requirements. The results of the research showed that majority of the household heads and their wives were 52-60 and 45-51 years old, respectively. Thirteen widows received RASTRA assistance and became household heads. The prioritised recipients were generally old widows based on the results of the village head's meetings with officials. The lowest level of formal education among most household heads and their wives was elementary school, with homemakers' low nutrition knowledge.

Household income dominantly ranged from Rp. 1,000,000.00 to Rp. 1,500,000.00 per month (about 63,80 US – 95,71 US per month) with 3-4 home members. The types of occupation of the household heads were divided into the following fields: a) on-farm, b) off-farm, and c) non-farm. The heads worked as landowners or farmers (44.6 percent), farm labourers (29.4 percent), traders, construction workers, and drivers (26.1 percent).

Most of the household heads in Pardasuka Sub-District were farmers and farm labourers. About 34.8 percent of household heads had side jobs and the majority worked as artisans, in addition to 30 percent of homemakers. An average of Rp. 561,639.00/month (about 35,83 US per month) was spent on food stuffs such as grains, animal foods, vegetables, and beans.

Poor households' coping mechanisms

Every food coping mechanism behaviour indicated a problem of household food insecurity, but it did not necessarily determine the severity. Therefore, each behaviour was grouped and given a scale value (scale values of 1, 2, and 3). Results showed that all households or respondents carried out food coping mechanisms with scale values of 1 and 2. The former was massively carried out by the households. According to Martianto (2006), actions with scale values of 1 and 2 are household adaptation stages for conducting food coping mechanisms.

The adaptation phase is initiated when household food insecurity is at low and moderate levels, and this stage is related to changing habits on obtaining and consuming foods. It happens at the early stage of the coping strategy.

The households undertook an average of seven food coping mechanisms with a scale value of 1. They were reducing the amount of foods consumed, buying cheaper foods, collecting wild foods, growing edible plants in their gardens or near their houses, changing the priorities on foods, and looking for side jobs. Besides mechanisms with the scale value of 1, the households also took on food coping mechanisms with a scale value of 2. They performed about four food coping mechanisms with this value, which were owing food stalls some money for what they took, taking money from their savings, changing food distribution, and borrowing money from relatives. Each food coping mechanism conducted by the households had a different period and frequency. The results also showed that 97.83 percent carried out food coping mechanisms with scale values of 1 and 2. Those performing mechanisms with scale values of 1, 2, and 3 simultaneously were only 2.2 percent. Food coping mechanisms with a scale value of 3 was migrating out of the island to get jobs due to limited number of jobs and low salaries in their places.

Figure 1 shows that two food coping mechanisms with a scale value of 1, which were buying cheaper foods and reducing the amount and variety of foods consumed, were applied to satisfy all households. For example, fish or chicken was replaced by *tempeh* and tofu, which prices were much lower. The number of households consuming rice with fish, chicken or others, and vegetables shrunk; they changed their menu to rice and vegetables only. Sometimes, some households consumed cassavas instead of rice. These two food coping mechanisms were carried out 2 times a week for a longer period, in line with Maxwell and Caldwell's (2008) study. The first step to overcoming food consumption undertaken by food-insecure households was to change their diet, whereby households were likely to divert food consumption from preferred to cheaper substitutes.

Another food coping mechanism conducted by majority of the households was obtaining wild leaves from the edges of rice fields and gardens for consumption. The wild leaves were taro leaves, cassava leaves, sintrong leaves

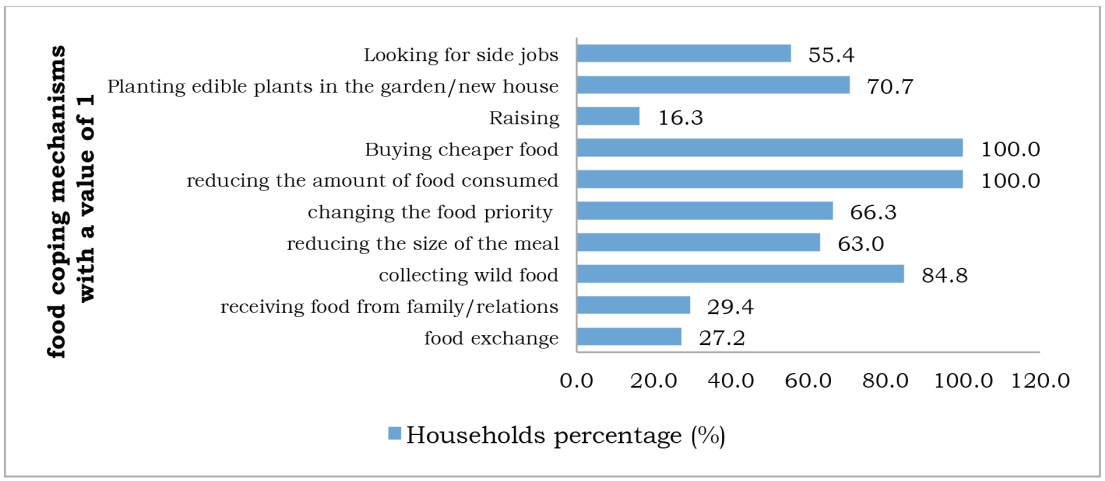

Figure 1. Percentages of household food coping mechanisms with a value of 1

(*gynura crepidioides*) etc. Approximately 70.7 percent of the households had gardens or yards for gardening, such as growing vegetables and tubers, like spinach, water spinach, tomatoes, chilli, eggplants, and cassavas. This action was consistent with the Sustainable Food Home Area Programme in Indonesia, known as *Kawasan Rumah Pangan Lestari* (KRPL), promoted by the Food Security Agency. The utilisation of a yard as a source of fulfilment of household food availability reduces food expenditure, improves Desirable Dietary Pattern (DDP), and supports household food security, especially in food-insecure areas. KRPL activities were also conducted to support government programmes for handling stunting and vulnerable food-insecure areas, developing border areas, and alleviating poverty (BKP of Ministry of Agriculture, 2019).

The food coping mechanisms with a scale value of 2 were chosen by the households when efforts to address food unavailability have been entirely unresolved. The addition of access to buy foods, changes in the distribution and frequency of meals, such as going through days without eating, were mechanisms with this scale value, and the percentages are shown in Figure 2.

The most common food coping mechanism with this scale value performed by the housewives was to owe the stalls some money for food stuffs, and it was usually carried out 2-3 times per month. Other actions included using savings to buy foods and changing its distribution. Changes in food distribution were carried out to reduce the portions of food consumed by the household members. Mothers did this to their children. For example, a mother who used to eat two ladles of rice ate only one ladle of rice instead as a manifestation of her change in food distribution. Also, some had their meals after the other members have had theirs and eaten as much as they wanted.

The last mechanism with the scale value of 2 was to pawn assets to make purchases; and the types of assets owned by the households were non-productive and productive. Non-productive assets were usually in the forms of electronic equipment, vehicles, savings, jewellery, and household appliances; while the productive ones were houses, lands, livestock, and rice fields or fields. In this case, households in the study area

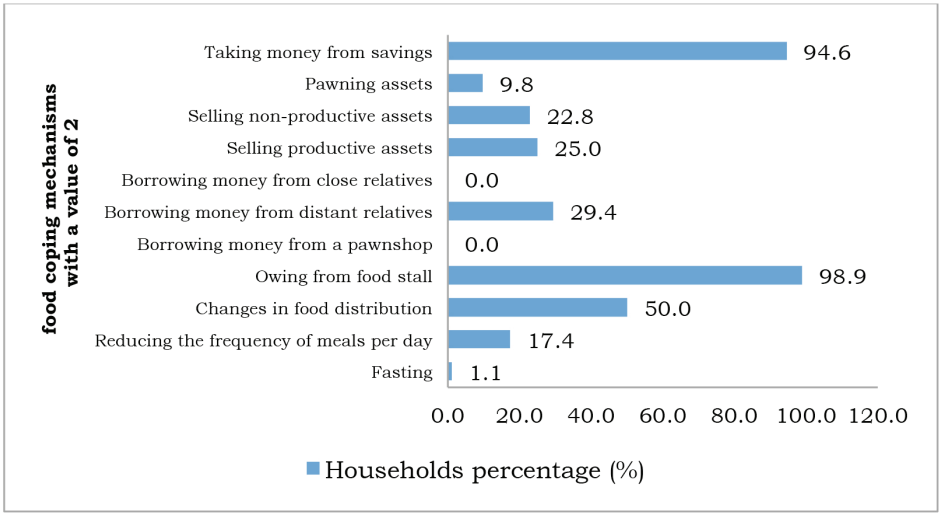

Figure 2. Percentages of household food coping mechanisms with a value of 2

tended to sell productive assets, such as livestock instead of non-productive ones. This was because the money from the sales of non-productive assets was significantly less and only met few needs. Furthermore, the lack of parties or traders willing to buy non-productive assets in the study area was another reason for why they preferred selling productive ones. Adepoju and Oyegoke (2018) stated that households with one form of asset or another had a higher likelihood of food security. Additionally, sales serves as a means of generating income to reduce food-related shocks. Farm households with access to credit are more likely to expand and diversify farming activities and buy inputs (Jabo *et al.,* 2017). Borrowing money for productive purposes is very important for farm households to increase their productivity.

The actors of the food coping mechanisms were household heads, housewives, and children. However, the study results showed that most food coping mechanisms were carried out by housewives, while homemakers played significant roles in almost all types of behaviours. They were the dominant actors of food coping mechanisms, especially those related to financial control. The households' decision-makers were the women because they managed resources to mitigate the short-term effects of not having enough food.

Dominant factors on food coping mechanism of poor households

The households performed food coping mechanisms due to the decrease or unavailability of foods, and their nature affected their access to the requirements. The factor analysis results showed two variables with an anti-image correlation value of lower than 0.5, namely the age of the housewives and the availability status of staple food (rice) (sufficient or less). According to Santosa (2012), when MSA variable value is less than 0.5, then it is unpredictable and unable to be further analysed. The correlation between variables of a factor should be strong enough, which is above 0.5. The variables of the homemaker's age and food availability status did not meet the statistical requirements. So, they were reduced and retested.

Results showed that the housewives undertook almost all the food coping mechanisms, and their age had no effect on the mechanisms because both young and old housewives carried out the process. Besides, they were not affected by the food availability status because majority had enough supplies due to the numerous ways of getting staple food (rice), such as the government's assistance (RASTRA), buying it from stalls, produce, and gifts from relatives. A household was classified as less efficient if they sold rice from the government to meet non-food needs, such as paying for electricity, settling debts, and buying various stuffs to cook, like vegetables and raw food materials.

The secondary analysis consisted of 19 variables with anti-image correlation values greater than 0.5, which allowed for further analysis. The KMO-MSA value was 0.67 and had a significance of 0.00 according to Bartlett's Test of Sphericity. This value indicated that the correlation between the variables and data can be a factor to analyse further, as shown in Table 1.

Table 1. Values of KMO and Bartlett's Test of Sphericity for food coping mechanism variables

Information	*Value*
Kaiser-Meyer-Olkin Measure of Sampling (KMO)	0.67
Bartlett's Test of Sphericity	658.42
Sig.	<0.001

Table 2. Eigen values and percentages of variance for six factors formed on food coping mechanisms of poor households

Factor	*Eigen value*	*Percentage of variance (%)*	*Cumulative variance (%)*
1	3.99	21.03	21.03
2	2.73	14.35	35.39
3	1.91	10.06	45.45
4	1.39	7.34	52.79
5	1.33	7.02	59.81
6	1.09	5.77	65.58

Six factors were formed with each an eigen value of greater than 1, with a total variance of 65.6. This value indicated that 65.6 percent of the poor households' food coping mechanisms were explained by these formed factors. Factor 1 contributed to 21.0 percent in forming the households' food coping mechanisms. The eigen values and variances for each factor are shown in Table 2.

Of the 19 analysed factors, six groups were formed, namely social conditions, food coping activities, economic conditions, assets, household head's occupation, and housewife's job. The dominant factor was social conditions, which included the age of the household heads, the length of their formal education along with the housewives, the nutrition knowledge of the housewives, and the number of actors involved in the food coping mechanisms with the scale value of 1. The factors determining the food coping mechanism of the poor households are shown in Table 3.

The length of household head's formal education had the highest loading factor value, 0.84. Loading factor is the correlation between a factor and a variable. The value indicated that the household head's length of formal education had a high correlation with the social condition factor in establishing the mechanisms to cope with mediocre food. The household heads' higher level of education resulted in the types of occupation and income leading to more purchasing power. The household heads' higher level of education helped them quickly understand new information, utilise available resources effectively and efficiently, and adopt innovations to improve food security (Boratynska & Huseynov, 2016). Therefore, the level of education increased the households' chance of becoming more food resistant without or with less coping mechanisms. This result is consistent with the study conducted by Onunka, Ihemezie & Olumba (2018), which found that the higher the education level of farmers, the less likely their ability to adopt several strategies to overcome food insecurity.

In the case of the dominant factor formed (factor 1), the housewife's formal education period was also part of the food coping mechanisms. A housewife's education affects her occupation and therefore her household income, as well as nutrition knowledge level. According to Damanik, Ekayanti & Hariyadi (2010), a wife's high education improves household welfare. Besides, high education also positively influences a homemaker's behaviour in managing the household, especially in the selection of daily foods, which accounts for all family members' nutritional status. A mother with higher nutrition education and knowledge enhances household food security and chooses appropriate food coping mechanisms, such as using yards for gardening and gathering

Table 3. Rotation values of factors on food coping mechanisms of poor households

No.	*Variable*	*Factor loading*	*Factor group*
1	Household head's age	-0.65	1 Social conditions
2	Formal education of household head	0.84	
3	Formal education of housewife	0.73	
4	Nutrition knowledge of housewife	0.58	
5	The number of actors involved in food coping mechanisms with a value of 1	-0.64	
6	Number of food coping mechanisms with a value of 1	0.65	2 Food coping activities
7	Number of food coping mechanisms with a value of 2	0.86	
8	Frequency of food coping mechanisms with a value of 1	0.63	
9	Frequency of food coping mechanisms with a value of 2	0.93	
10	Household food security status	-0.68	3 Economic conditions
11	The number of household members	0.71	
12	Income	0.55	
13	Food expenditure	0.65	
14	The number of actors involved in food coping mechanisms with a value of 2	0.54	
15	Assets	0.84	4 Assets
16	Quantity of rice for a week	0.76	
17	Household head's job	0.72	5 Household heads' job
18	Household head's side job	0.64	
19	Housewife's job	0.84	6 Housewives' job

edible wild plants to reduce expenditure and increase household consumption diversity.

The household heads were also one of the variables undertaking food coping mechanisms, with the majority within the age range of 52-60 years and older than 60 years of age. However, in their old age, they experience a decrease in endurance, health, and suffer various psychological stresses. Furthermore, their functions of organs and ability to work have reduced, which shrunk their income and food purchasing power.

A number of food coping actors undertook mechanisms that fell in the scale value of 1 in poor households. The mechanisms conducted by the households when food shortage occurred encompassed activities for increasing income, changing eating habits, and getting immediate access to foods. According to Gazuma (2018), activities generating income and food are negatively related to food insecurity. The food coping mechanisms with the scale value of 1 were done by 3 actors of the household members, while those with the scale values of 2 and 3 were done by 1-2 actors. The food coping mechanism actors were usually the father or husband, mother or wife, and children. More members were normally involved in the mechanisms to increase income, provide more opportunities for food availability, and overcome food

shortage. Maxwell and Caldwell's (2008) stated that the more people participated in overcoming food-related issues, the less food insecurity there was.

These findings implied that education is a determinant which affects the food coping mechanisms performed by both husbands and their wives in poor households. It also affects nutrition knowledge and types of occupation that, in turn, influence the income earned by the households. Besides food aid (RASTRA), poverty alleviation programmes involving increasing access to education in poor rural households are a viable solution to mitigate food insecurity.

CONCLUSION

In conclusion, strategies adopted by most households during the food shortage were to buy cheaper foods and to reduce the amount and variety consumed. The dominant factor affecting food coping mechanisms of poor households was social condition. This included the household head's age, length of formal education, as well as his wife's nutrition knowledge, and the number of actors in the mechanism. Formal education has the most significant influence on establishing a survival mechanism to overcome food insecurity at the household level.

Education had the highest correlation with food coping mechanism and was the primary variable adopted by poor households to overcome limited access. The government and other related parties should optimise formal and non-formal education variables, especially for housewives as the dominant actor of food coping mechanisms. With all that in mind, it is safe to infer that increasing public knowledge will enhance communal food security.

Acknowledgement

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Authors' contributions

WDS, principal investigator, conceptualised and designed the study, prepared the draft of the manuscript and reviewed the manuscript; WAZ, conducted the study, data analysis and interpretation; TSS, led the data collection; AM, assisted in drafting of the manuscript and reviewed the manuscript.

Conflict of interest

All authors have read, looked at, and agreed to the content of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under consideration for publication elsewhere.

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Application of STRONGkids method on assessing the risk of malnutrition among hospitalised children in Universitas Sebelas Maret Hospital

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ABSTRACT

Introduction: Hospital malnutrition (HM) in children augments morbidity and mortality, thus early detection is a preventive measure that may improve a patient's condition. This study identified the risk factors of HM among children hospitalised in Universitas Sebelas Maret (UNS) Hospital using the STRONGkids method. **Methods**: This observational analytical cross-sectional study was performed in children hospitalised at the paediatric ward of UNS Hospital between February and August 2021. The samples were taken by consecutive sampling technique. Subjects meeting the inclusion criteria were assessed for their underlying disease, nutritional status based on World Health Organization Anthro software, and STRONGkids score. Chi-square test and logistic regression analysis were used, with a *p*-value of <0.05 considered as statistical significance. **Results**: A total of 173 children were included in the study, 56% were males, mean age was 62.5 months, 45% had an underlying chronic disease, and 17.9% experienced moderate malnutrition. Based on the STRONGkids score, 39.3% children were at high risk of malnutrition and 60.7% were at moderate risk of malnutrition. Logistic regression analysis showed that high risk of HM was significantly associated with age (OR 2.58, 95% CI 1.38-4.84, *p*=0.003), chronic disease (OR 7.23, 95% CI 3.3-15.86, *p*=0.018), and moderate malnutrition (OR 13.5, 95% CI 3.96-45.98, *p*<0.001). **Conclusion**: Children hospitalised in UNS Hospital were at risk of malnutrition. Toddlerhood, chronic disease, and moderate malnutrition significantly increased the risk of HM. Thus, these children need optimal nutritional support to improve their clinical condition. STRONGkids is a convenient and easy method to identify malnutrition risk during hospitalisation.

Keywords: children, hospital malnutrition, nutritional status, STRONGkids

INTRODUCTION

Malnutrition in children remains a serious problem globally, both in developed and developing countries. The World Health Organization (WHO) reports that children under five years of age face nutritional problems, in which 150.8 million are stunted and 50.5 million are underweight (WHO, 2018). Malnutrition can occur either prior to a hospitalisation

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or during hospitalisation. Hospital malnutrition (HM) is malnutrition seen in hospitalised patients, which are characterised by weight loss >2% in <7 days of treatment or 5% in 8-30 days of treatment or 10% in >30 days of treatment (Sidiartha, 2008; Gouveia & Silva, 2017). The prevalence of HM in paediatric patients is relatively high. It varies considerably, ranging from 2.5 to 51% based on the population and the operational definition used (McCarthy *et al.*, 2019; Nasar *et al.*, 2014).

HM increases morbidity, mortality, disease complications, length of stay (LOS), and treatment costs (Freijer *et al.*, 2018). The course of the disease, hospital environment, and various medical procedures performed on paediatric patients cause stress to the patients and their parents. This results in reduced or loss of appetite leading to decreased nutritional intake (Maryani *et al.* 2016). Meanwhile, ill children experience hypermetabolism, malabsorption, and even loss of nutrients, which impact the increased nutritional requirement. Therefore, inadequate nutrition during hospitalisation will cause HM. Early detection of HM is crucial and beneficial for patients, doctors, and hospital management as it may prevent complications and reduce hospital costs (McCarthy *et al.* 2019).

One of the methods to detect early HM is the Screening Tool for Risk of Impaired Nutritional Status and Growth (STRONGkids) (Gouveia, Tassitano & Silva, 2018). This method is straightforward and practical, and can be carried out by nutritionists, nurses, medical students, and general practitioners. It has been developed in Europe and is the most often validated nutritional screening tool to identify the risk of HM (Pérez-Solís *et al.*, 2020). Therefore, we performed this study to define the risk factors influencing HM using the STRONGkids method and evaluate its correlation with nutritional status, underlying disease, sex, and age of children hospitalised in the paediatric ward of UNS Hospital, Sukoharjo, Indonesia.

MATERIALS AND METHODS

An observational analytical cross-sectional study was conducted in children hospitalised at the paediatric ward of UNS Hospital from February to August 2021. This study was approved by the Public Health Research Ethics Commission of Dr Moewardi Hospital, Surakarta, Indonesia, with the approval number of 1.219/X/HREC/2020. A consecutive sampling technique was applied for sample collection. Patients aged 1 month to 18 years old were included in this study. Sample size was calculated using OpenEpi software to obtain a representative sample, with a minimum sample size of 158–173 children. Sample size calculation took into account the average of hospitalised children, the prevalence of high/moderate nutrition score based on previous reports, and possibility of loss to follow-up (Maharani *et al.*, 2020; Tuokkola *et al.*, 2019; Dean, Sullivan & Soe 2013). We excluded those discharged before 24 hours, dead within 24 hours of arrival, on ventilator assistance during hospitalisation, or in which the nutrition screening tool could not be applied within the first 72 hours of admission. Parents or guardians who agreed to participate in this study signed an informed consent during the hospital stay. We interviewed the parents or guardians regarding the history of patients' underlying disease.

The collected data were age, sex, underlying disease, nutritional status, and STRONGkids score. These data were taken at admission. Acute or chronic diseases were diagnosed by paediatricians blinded to the study based on each disease's guidelines. In this

study, acute diseases were defined as diseases currently affecting the patients leading to hospitalisation, for example Dengue Haemorrhagic Fever (DHF), typhoid fever, diarrhoea, pneumonia, and febrile seizure. Chronic diseases were described as prolonged underlying diseases suffered by subjects, like autoimmune, malignant, and congenital diseases. Nutritional status of patients was determined based on weight-for-height *z*-score for subjects <60 months old using the WHO Anthro software and body mass index-for-age *z*-score for the older ones. STRONGkids is a nutrition risk screening tool, comprising four items i.e. (i) subjective clinical assessment of children's nutritional status, (ii) history of nutritional status based on weight gain, (iii) acute condition affecting nutritional status such as excessive diarrhoea or vomiting in the last three days, reduced food intake, as well as inability to have adequate food intake due to pain, and (iv) weight loss resulting from underlying chronic conditions. Based on STRONGkids, study subjects were categorised as high risk of HM if the score was 4-5 points, moderate risk if the score was 1-3 points, and low risk if the score was 0 point.

All demographic data were presented in percentages. Chi-square test was used for categorical variable analysis. Risk factors were presented as odds ratio (OR) with a 95% confidence interval (CI). Logistic regression analysis was performed to obtain significant risk factors for HM with a significance level of $p<0.05$. All data were statistically analysed using SPSS Statistics for Windows version 25.0 (IBM Corp, Armonk, New York, USA).

RESULTS

The demographic characteristics of subjects were a general description of the entire sample obtained in this study. All children hospitalised in UNS hospital were at risk of malnutrition. Among these study subjects, moderate risk of HM was more common than high risk (Table 1). Most children hospitalised at UNS hospital were well-nourished. However, based on STRONGkids classification, they had a moderate risk for HM. In our study, paediatric patients with either acute or chronic disease had a risk of HM based on STRONGkids classification. Subjects with chronic disease had a higher risk of HM than those with acute disease (Table 2).

Table 1. The demographic characteristics of study subjects

Characteristics	*n (%)*
Sex	
Male	97 (56.1)
Female	76 (43.9)
Age (months), mean (*SD*)	79.8 (62.5)
Toddler (< 60 months)	88 (50.9)
≥ 60 months	85 (49.1)
STRONGkids	
High risk	68 (39.3)
Moderate risk	105 (60.7)
Underlying disease	
Chronic	78 (45.1)
Acute	95 (54.9)
Nutritional status	
Severe malnutrition	6 (3.5)
Moderate malnutrition	31 (17.9)
Well-nourished	136 (78.6)

Nutritional status and underlying disease significantly affected STRONGkids score ($p=0.018$ and $p<0.001$, respectively). Surprisingly, well-nourished paediatric patients also had a high risk of HM (52.90%). Nutritional status played a significant role in HM based on STRONGkids score (OR 13.89; 95% CI 1.57-122.90). Study subjects with acute underlying disease were at moderate risk of HM (74.3%). Both subjects with chronic and acute diseases were at risk of HM, either high

Table 2. STRONGkids score on nutritional status and underlying disease of study subjects

Variable	*STRONGkids*		*OR*	*95% CI*	*p*
	High risk	*Moderate risk*			
Nutritional status			13.89	1.6-122.9	0.018
Severe malnutrition	5 (7.4)	1 (1.0)			
Moderate malnutrition	27 (39.7)	4 (3.8)			
Well-nourished	36 (52.9)	100 (95.2)			
Underlying disease			8.67	4.3-17.5	<0.001
Chronic	51 (75.0)	27 (25.7)			
Acute	17 (25.0)	78 (74.3)			

or moderate risk (OR 8.67; 95% CI 4.30-17.50) (Table 2). Logistic regression analysis revealed that age, sex, underlying disease, and nutritional status were risk factors of HM based on STRONGkids score classification. Children at toddler age were at significant risk of HM, and so were those with chronic disease and moderate malnutrition. Toddlers were at 2.58 folds risk of suffering from HM than older aged children. Meanwhile, children with chronic disease had 7.23 times higher risk of experiencing HM than those with acute disease. In addition, children with moderate malnutrition had 13.50 folds risk of having HM (Table 3).

DISCUSSION

In this study, we found high proportions of HM, with 39.3% of children at high risk and 60.7% at moderate risk of malnutrition. HM was associated with younger age, chronic disease, and moderate malnutrition. Several studies have reported that boys have a higher risk of HM than girls. However, another study reported that girls dominated HM (80%) (Juliaty, 2013). To date, there has not been any study explaining the factors that cause gender dominance (Hafsah, Prawitasari & Djais 2019).

Age is an essential risk factor for malnutrition. Malnutrition in children under five years is caused by a complex combination of food and healthcare service availability, accessibility, and utilisation (Govender *et al.*, 2021). This current study revealed that HM was more common in toddlers (50.9%). A previous study reported that age <5 years old was a risk factor of HM in children hospitalised in a rural hospital (Prasetya, Haryanti & Nurani, 2021). Similarly, another study found that children under 60 months old were more likely to suffer from hospital-acquired malnutrition (Spagnuolo *et al.*, 2013). Age is also a risk factor for weight loss during hospitalisation in children and adolescents. Many factors can influence this, including changes in the underlying condition, management, diet, and mental stress. A study by Rocha, Rocha & Martins (2006) in Brazil reported that 51.60% of children aged <5 years old

Table 3. Logistic regression analysis on STRONGkids for all variables

Variable	*Exp(B)*	*95% CI for Exp(B)*	*p*
Age: toddler	2.58	1.38 – 4.84	0.003
Sex: male	1.11	0.51 – 2.47	0.368
Underlying disease: chronic	7.23	3.3 – 15.86	0.018
Nutritional status: moderate	13.5	3.96 – 45.98	<0.001

lost weight on discharge. Protein-energy malnutrition (PEM) in children <5 years old remains one of the most serious public health problems in developing countries. It has been estimated that 80% of these malnourished children live in Asia, 15% in Africa, and 5% in Latin America. In addition, 43% of these children (230 million) are chronically malnourished (Rocha *et al.*, 2006). Children under five tend to develop HM since weight loss is the only factor taken into account in assessing nutritional status of hospitalised children (Teixeira & Viana, 2016). Children under the age of five require more calories per kilogram of body weight than older children and adolescents, which puts them at risk for HM. Patient's condition, research context, type of hospital, country status, and age group determine the prevalence of HM in each study (Gouveia & Silva, 2017; McCarthy *et al.*, 2019).

Underlying disease and nutritional status were significant risk factors for the occurrence of HM in our study subjects. These findings support a previous study conducted in several hospitals in Bali and West Borneo, which reported that a high risk of HM was significantly associated with chronic, moderate-to-severe disease and nutritional status (Sidiartha, 2018; Prasetya, Haryanti & Nurani, 2021). Malnutrition is a pathological condition affecting most children. Children are more likely to become malnourished because they demand more energy for growth and have fewer energy reserves. Based on our data, malnutrition and chronic illnesses in children caused the most significant weight loss. Campanozzi *et al.* (2009) revealed that malnourished children had a lower BMI at discharge than those who were not malnourished at admission. Weight loss during hospitalisation relates to the primary ailment and reason for admission as fast catabolism of lean body mass occurs due to inflammatory response. Inflammation increases nitrogen excretion and basal energy consumption, leading to weight loss. Malnutrition can be aggravated by a chronic condition resulting in protein catabolism, lower energy intake, and increased energy expenditure (Gouveia & Silva, 2017). Fever, anorexia, vomiting, and diarrhoea further exacerbate the imbalance in intake and nutritional needs (Campanozzi *et al.*, 2009). Malnutrition affects the immune system, increasing the risk of surgical complications, infection, and poor wound healing. Hence, malnutrition impairs recovery, prolongs hospital stays, and increases costs and other healthcare-related factors (Mehta & Duggan, 2009). Most studies confirm that every hospitalised child is at risk of malnutrition. As a result, professionals should be aware of the risk of malnutrition in hospitals (Quadros *et al.*, 2018).

Based on the obtained STRONGkids scores, all subjects in this study were classified as having high risk (39.3%) or moderate risk (60.7%) of HM. Other studies in Romania and Brazil demonstrated that the risk for malnutrition in children admitted to hospitals assessed with STRONGkids score was lower than our study (Mărginean *et al.*, 2014). The differences are perhaps due to the interpretation of each element of the question in the STRONGkids method. Screening for malnutrition during hospitalisation is intended to identify the risk of HM. A high STRONGkids score reliably predicts the risk of malnutrition (Borda, Espitia & Otalvaro, 2018). In our hospital, we use STRONGkids for malnutrition screening because it is an easy to apply, simple, and accurate instrument. In paediatric in-patients, routine screening for the risk of malnutrition is critical in recognising at-risk children who need dietary intervention (Maharani *et al.*, 2020). All children at risk of HM require comprehensive nutritional

intervention, extended hospital stays, and high hospital costs. They also have an increased risk of mortality (Sidiartha 2008; McCarthy *et al.*, 2019). Therefore, we must be more aware of nutritional care.

The most outstanding independent risk factor for HM in our hospital was patients with moderate malnutrition. McCarthy *et al.* demonstrated that at the time of discharge, patients who were malnourished at the time of admission lost more BMI than those with better nutritional status at admission (McCarthy *et al.*, 2019). Our study found that subjects with moderate malnutrition had a 13.50 folds risk of suffering from HM. These findings suggest that STRONGkids can be used to detect malnutrition risk in the hospital before it progresses to more severe malnutrition, which will be increasingly challenging to seek a cure for the underlying disease (Beser *et al.*, 2018; Pérez-Solís *et al.*, 2020). Another study also reported that the STRONGkids screening method was associated with malnutrition at the time of hospital admission (Moeeni, Walls & Day, 2014). A study in China reported that hospitalised paediatric patients evaluated using STRONGkids were at high risk of malnutrition, especially those with heart disease, respiratory disease, oncological disease, and underlying gastrointestinal disease (Cao *et al.*, 2014). Paediatric patients who have moderate or high risk of malnutrition should be monitored closely during hospitalisation so that early intervention can be carried out to improve their prognosis.

We found a correlation between STRONGkids scores with chronic disease, as well as nutritional status. Children with chronic diseases tended to have higher STRONGkids scores than those with acute illnesses. Children with chronic disease have a 7.23 times risk of experiencing malnutrition. Other studies also suggested that children with chronic diseases have a higher risk of malnourishment during hospitalisation (Sidiartha, 2018). A study in China reported that the STRONGkids score was closely related to patient's clinical symptoms. Children at high risk of malnutrition have more complications, longer treatment duration, more significant weight loss and nutritional support, and higher hospital cost (Cao *et al.,* 2014). Children at risk of malnutrition need adequate and optimal nutritional intake during hospitalisation to support the healing process of their underlying disease (Beser *et al.,* 2018). Another study stated that nutritional support in children under three years of age with congenital heart disease reduced LOS and mortality after surgery. Although nutritional support during hospitalisation has been shown to reduce disease complications, the nutritional aspect is still underestimated (Bauer, Jürgens & Frühwald, 2011). During hospitalisation, the concern is mostly on the primary medical problem, so often times, the importance of nutritional management is neglected. According to Kazem & Hassan (2008), the nutritional condition of children had a significant impact on those who were either well-nourished or mildly malnourished at the time of admission. Therefore, increasing paediatric nutritional care according to underlying disease is an effort to reduce the incidence of malnutrition in hospitals.

Our study had several limitations. The assessment of nutritional status was primarily based on basic anthropometric measurements (weight and height), with no consideration given to other indicators such as skinfolds or body composition, which may have resulted in subjects being miscategorised as malnourished, especially in the case of chronic malnutrition. In addition,

many other risk factors such as parental characteristics, socioeconomic conditions, the type and volume of nutritional intake during hospitalisation were not studied, which may affect the prevalence of HM. Therefore, future studies must consider all the aforementioned factors and involve multiple centres so that the findings can be generalised.

CONCLUSION

All children hospitalised in UNS Hospital were at risk of malnutrition. Toddlerhood, chronic disease and moderate malnutrition significantly increased the risk of HM. Hence, optimal nutritional support is necessary for these children to improve their clinical condition. The STRONGkids method is convenient and easy for screening the risk of malnutrition during hospitalisation, which health workers can apply.

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Authors' contributions

MGKS, principal investigator, conceptualised and designed the study, conducted the study, performed data analysis and interpretation, prepared the draft of the manuscript, and reviewed the manuscript; VW, reviewed the manuscript and advised on the data analysis, as well as interpretation; ASP, led the data collection, analysed the nutritional status, and reviewed the manuscript; SW, assisted in drafting of the manuscript and reviewed the manuscript; AML, led the data collection and analysed the nutritional status.

Conflict of interest

The authors have no conflict of interest in the research, authorship, and publication of this work. None of the authors received funding from the government, business sector, or non-profit organisation.

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REVIEW

Regulatory status of bioactive non-nutritional food components in Southeast Asian countries

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ABSTRACT

A review conducted on the regulatory status of bioactive non-nutritional food components in foods and beverages in eight Southeast Asian countries indicates these components have been recognised for their health benefits. Indonesia and Malaysia have promulgated specific regulations allowing the addition of these bioactive components in foods and beverages, provided a list of the permitted components that may be used, and clear process for the industry to apply for new components. Both countries also have separate regulations that govern the making of function claims which refer to the beneficial physiological or health effects brought about by such bioactive components. The other six countries do not have a specific regulation governing the use of these bioactive components. However, these countries also permit the making of similar function claims, provided they are scientifically substantiated, preferably human clinical trials. Each country has slightly different requirements and process in place for reviewing applications for claims. All countries, except Myanmar, also allow the sale and marketing of foods containing probiotics, another functional food component. Indonesia, Malaysia, Philippines and Thailand have promulgated specific probiotic regulations and, except for Indonesia, have published permitted list of probiotics. All seven countries have provisions for the industry to apply for the use of new probiotics. Malaysia, Philippines, Singapore and Thailand permit the use of a pre-approved generic function claims related to probiotics. The sharing of experiences in regulatory approaches would be beneficial to the advancement of scientific and regulatory development of bioactive non-nutritional food components in the region and would benefit all stakeholders.

Keywords: bioactive food components, food regulations, functional components, non-nutritional components

INTRODUCTION

Nutrients, as defined by Codex Alimentarius, are substances normally consumed as a constituent of food which provide energy and which are needed for growth, development and maintenance of life. A deficit of nutrients may cause characteristic biochemical or physiological changes to occur (FAO/WHO, 2017). The traditional major groups of nutrients in foods come to mind, namely carbohydrates, fats, proteins, vitamins, minerals and water.

Foods also contain non-nutritional food components or constituents that are biologically active compounds. They include a variety of components of plant and animal origin and have been

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known by various names as well as a number of live lactic acid bacteria that have been recognised as probiotics. All these bioactive non-nutritional food components have been shown through many epidemiological and clinical studies to be able to serve physiological roles beyond meeting basic nutritional requirements and may possess health-enhancing properties. They have been generally recognised as bioactive or functional components (Tee, Wong & Chan, 2021).

Recognising the potential beneficial health effects, regulatory authorities in some countries in the region have established regulatory framework to allow the addition of bioactive non-nutritional functional food components to foods and beverages. Permission is often given on a case-by-case basis, upon submission of applications supported by scientific data. Some countries also permit these components to make health claims, specifically "other function claims", as defined by Codex Alimentarius guideline (FAO/WHO, 2013), if they meet the required regulatory criteria.

However, the regulatory status on the use of these bioactive components or constituents and the health claims permitted to be made in relation to these components in Southeast Asian (SEA) countries is unclear and not documented. A review was thus conducted to document the regulatory status of the addition of bioactive non-nutritional food components to foods and beverages (hereafter in this publication referred to as foods). Such information may serve as:

- useful information for food companies in their innovation programmes to add bioactive non-nutritional food components to improve the nutritional profile of their products;
- reference for regulators for the introduction of a regulation to permit the addition of bioactive non-nutritional food components and the associated other function claims
- basis for discussion for harmonisation of the use of bioactive non-nutritional food components or the regulatory review framework governing these components among countries in the region.

This publication documents findings obtained from the review.

METHODOLOGY

A survey template was prepared by the authors to obtain information regarding regulatory control of bioactive non-nutritional food components in SEA countries. The key information in the template include the following two broad topics:

1. Regulatory status of use of bioactive non-nutritional food components, including information on whether these components are permitted to be added to foods, availability of specific regulation, if a positive list of permitted such food components is published and requirements and review process for application for use of such bioactive food components;
2. Regulatory status of permitted health claims related to bioactive non-nutritional food components, and if permitted, the application requirements and review process for such health claims.

The official regulations or documents related to the use of bioactive non-nutritional food components were obtained from regulatory authorities and their respective websites. Pertinent information were extracted from these documents. Where necessary, officials and experts from regulatory authorities

were consulted on specific aspects of the survey and requested to assist in providing and verifying the information in the template. The entire review including verification of information with regulatory experts was carried out from mid 2020 till end 2021. During preparation of the manuscript in 2022, effort was still made to check for updates in the regulations and guidelines.

Information on the use of bioactive non-nutritional food components in the Codex Alimentarius system, specifically in relation to health claims, is also obtained for inclusion in this publication. It is well recognised that guidelines and standards from the Codex system serve as references to countries in the development of national regulations.

RESULTS

The required information were only obtained from eight of the ten countries in SEA, namely Brunei Darussalam, Indonesia, Malaysia, Myanmar, Philippines, Singapore, Thailand and Vietnam. No response was obtained from Cambodia and Laos regulatory authorities and no relevant documents were obtainable from the official websites of these organisations.

Results of the survey are presented in accordance to the two broad topics mentioned above, that is in relation to the use of bioactive non-nutritional food components in foods and associated health claims. Before presenting findings from the eight SEA countries, the status in Codex Alimentarius is first summarised. Within each of the topics, the status in each country is separately presented.

Regulatory framework for the addition of bioactive non-nutritional components to foods and beverages

Table 1 summarises how Codex framework and authorities in eight SEA countries regulate the use of bioactive non-nutritional components in foods and beverages. Codex framework and all countries have made provisions for the use of these food components in foods. However, the regulatory approaches in Codex and each of these countries vary considerably, details of which are summarised in the following paragraphs.

Codex Alimentarius

There is no specific document within the Codex system that provides guideline on the use of bioactive non-nutritional food components, such as within the context of this review. However, the Codex framework does recognise the positive roles that bioactive non-nutritional components in foods may play in human health. This is evidenced from the provisions made for health claims to be made on "food constituents" in the Codex Guidelines for Use of Nutrition and Health Claims CAC/GL 23-1997 (FAO/WHO, 2013). In this Guidelines, health claim is defined as *any representation that states, suggests, or implies that a relationship exists between a food or a constituent of that food and health.* Health claims include the following: (1) nutrient function claim; (2) other function claim, and (3) reduction of disease risk claim. The three claims refer to different relationship between a food or its constituents and various physiological functions or health effects, as defined below:

(1) *Nutrient function claims describe the physiological role of the nutrient in growth, development and normal functions of the body.*

(2) *Other function claims describe specific beneficial effects of the consumption of foods or their constituents, in the context of the total diet on normal functions or biological activities of the body. Such claims relate to a positive contribution to health or to the improvement of a function or to modifying or preserving health.*

Table 1. Summary of regulatory framework for addition of bioactive non-nutritional food components in eight SEA countries

	Specific regulation or guideline	*Terminology used*	*Positive list of permitted bioactive components*	*Application for use of new bioactive components*
Codex Alimentarius	No specific Codex guide on the use of bioactive components. However, Codex guideline on claims has made provisions for health claims to be made on "food constituents" which is to be differentiated from role of nutrients in nutrient function claim	Main term is "food constituent"	No positive list	Not applicable
Brunei Darussalam	No specific regulation permitting use of specific bioactive components or probiotics	Various terms used, including novel ingredients, food ingredients, lactic acid producing bacteria	No positive list of permitted bioactive components and probiotics	No specific framework for applications which will be assessed on a case-by-case basis
Indonesia	Regulation 30 of 2021 on Requirements for the Addition of Nutrients and Non-nutritional Substances to Processed Foods; Regulation 1/2022 on Monitoring of Claims on Processed Food Label and Advertising permits use of probiotics in processed foods	The specific term used in regulation is "non-nutritional substances" and include compounds or bioactive/ functional components found in food that do not function as nutritional substances but affect health. The term probiotics is in accordance with FAO/WHO 2001 consultation report definition	Positive list of 16 non-nutritional substances in Regulation 30/2021. No positive list of permitted probiotics	Applications for use of new non-nutritional substances and probiotics can be made to the authorities accompanied by safety and scientific data. Regulatory framework established to review applications.

Table 1. Summary of regulatory framework for addition of bioactive non-nutritional food components in eight SEA countries (cont'd)

	Specific regulation or guideline	*Terminology used*	*Positive list of permitted bioactive components*	*Application for use of new bioactive components*
Malaysia	Regulation 26 Added Nutrients permits use of other food components and Regulation 26A regulation provides for use of probiotics in foods and beverages	The specific term used in regulation is "other food components", listed as a group of non-nutritional components in the list of permitted added nutrients. The use of the term Probiotics is in line with FAO/WHO 2001 consultation report definition	Listed in Table I of the Twelfth Schedule of Regulation 26 lists a total of 25 other food components, 11 of which are various forms of dietary fibre. Table in Regulation 26A lists 32 permitted strains of probiotics	Applications for new other food components and probiotics can be made using a prescribed application forms. Regulatory framework established to review applications, require scientific data to demonstrate safety and beneficial effects
Myanmar	No specific regulation permitting use of specific bioactive components or probiotics	Various terms used, including ingredients of known food sources such as plant/botanical, animal and microbial including their metabolites, derivatives or enzymes	No positive list of permitted bioactive components and probiotics	No specific framework for applications which will be assessed on a case-by-case basis; data on safety and specific health benefits to be provided
Philippines	No specific regulation for the use of bioactive non-nutritional components. BFAD Circular No. 16s 2004 provides for use of probiotics in foods	Various terms used, including "any substances" and "dietary ingredients". Bioactive substances like flavonoids, carotenoids, antioxidants, phytochemicals, lycopene, fatty acids, peptides are collectively termed as "any substance". The use of the term Probiotics in BFAD Circular No. 16s 2004 is in line with FAO/WHO 2001 consultation report definition	No positive list of permitted bioactive components. BFAD circular No. 16s 2004 published a list of 5 genera/species of microorganisms to be used as probiotics	Applications for new bioactive components may be made through product registration under Administrative Order 2014-0029 through an online process; no prescribed form. Applications for new probiotics can be made using electronic registration system under FDA Circular 2016-014

Table 1. Summary of regulatory framework for addition of bioactive non-nutritional food components in eight SEA countries (cont'd)

	Specific regulation or guideline	*Terminology used*	*Positive list of permitted bioactive components*	*Application for use of new bioactive components*
Singapore	No specific regulation for the use of bioactive non-nutritional components or probiotics.	Various terms used, including food ingredients, novel foods. The use of the term Probiotics is in line with FAO/WHO 2001 consultation report definition	No positive list of permitted bioactive components and probiotics	In most cases, the use of bioactive food components is tied up with other function claims - see next section on health claims. Applications for new probiotics must be accompanied by safety and scientific data
Thailand	No specific regulation for the use of bioactive non-nutritional components. Use of probiotics in foods is permitted under two Notifications of the Ministry of Public Health: No. 339, B.E. 2554 (2011) and No. 346, B.E 2555(2012)	Various terms used, including food ingredients, novel ingredient. The use of the term Probiotics is in line with FAO/WHO 2001 consultation report definition	No positive list of permitted bioactive components. MoPH notification No. 339 has published a list of 23 species of microorganisms to be used as probiotics in foods	Applications for use of bioactive non-nutritional food components is linked to applications for other function claims (see next section on health claims). Applications for new probiotics are via an online system, accompanied by data on safety functional role
Vietnam	No specific regulation for the use of bioactive non-nutritional components or probiotics in foods. However, ingredients with biological activities may be added to specific supplemented foods under MOH Vietnam Circular 2014.	Various terms used, including "healthful components for the body" and biologically active substances. Probiotics is one of the permitted "healthful components"	No positive list of permitted "healthful components for the body" and probiotics	No specific framework for consideration of applications for the use of any food components/ constituents that they intend to add to supplemented foods. Application to register a new bacteria as a probiotic can be made under supplemented food, supported by safety data

(3) *Reduction of disease risk claims refer to claims relating the consumption of a food or food constituent, in the context of the total diet, to the reduced risk of developing a disease or health-related condition.*

There is a clear distinction between claims that may be made on nutrients versus food constituents. Nutrient function claim refers to relationship between conventionally recognised nutrients (e.g. vitamins, minerals, protein) with their physiological functions. On the other hand, other function claim and reduction of disease risk claim make reference to the effect of "consumption of foods or their constituents" and health.

In the annex of this guideline which provides recommendations on the scientific substantiation of health claims, the process for substantiation include identifying the "proposed relationship between the food or food constituent and the health effect". A few examples of such food constitutents given in the annex are plant sterols, fibres and lactic acid bacteria. It is clear that the food constituents in these Codex health claims refer to non-nutritional food components that are bioactive and may bring about positive health effects.

With regard to the use of probiotics in foods and beverages, there is currently no specific Codex text on this. Back in 2001, there were two expert consultations organised by Food and Agriculture and Organization (FAO) and World Health Organization (WHO), the parent organisations of Codex Alimentarius (FAO/WHO, 2001; FAO/WHO, 2002). In the 2001 consultation, a definition of probiotics was proposed and among the various recommendations, it was felt that the regulatory status of probiotics as a component in food has to be established on an international level.

In this regard, there is a currently a proposal to develop a harmonised guideline on probiotics within the Codex system. It was last discussed in the 41st session of the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) in 2019 and is expected to be re-tabled for discussion at the 43rd session of CCNFSDU in March 2023 (FAO/WHO, 2019).

Brunei Darussalam

The Food Safety and Quality Control Division (FSQCD) of the Ministry of Health has been the food safety regulatory authority in Brunei Darussalam. In a recent development, the Brunei Darussalam Food Authority (BDFA) was established on 1 January 2021 to strengthen and develop a robust food safety and quality system in the country (official website: https://www.moh.gov.bn/SitePages/Food%20Safety%20and%20Quality%20Control%20Division.aspx).

There is no specific regulation permitting the use of bioactive non-nutritional food components in foods/beverages under the Public Health (Food) Regulations (R1, chapter 182) (MOH Brunei, 2001). However, if there is an interest to use a specific ingredient, the industry may make enquiries to the BDFA, which will assess each application on a case-by-case basis.

In addition to the above, there are regulations permitting the use of yet another category of functional food component, namely lactic acid producing bacteria in foods. Foods containing these bacteria are milk drink or cultured milk drink (Regulation 139), sour cream (Regulation 150), and yoghurt (Regulation 162).

Novel ingredients or any ingredients not covered in the current legislation are reviewed administratively on a case-by-case basis. Importers may enquire about the suitability of an ingredient

to the BDFA. The assessment duration varies depending on the ingredient in question. In most cases, the ingredient is advised to follow the requirements under Codex Alimentarius standards.

Indonesia

The food control authority in Indonesia is the National Agency of Drug and Food Control or Indonesian Food and Drug Authority (Indonesian FDA) (official website: https://www.pom.go.id/new/home/en).

Regulation 30 of 2021 by the Indonesian authorities recently updated the specific regulation that spells out the Requirements for the Addition of Nutrients and Non-nutritional Substances to Processed Foods (FDA Indonesia, 2021). Nutrients are defined in the said regulations as substances or compounds contained in food consisting of carbohydrates, dietary fibre, protein, fat, vitamins, minerals, water, and other components that are beneficial for human growth, development and health. At the same time, the regulation has stated that non-nutritional substances are compounds or bioactive/functional components found in food that do not function as nutritional substances but affect health.

Appendix 1 of the said regulation has provided a positive list of 16 non-nutritional substances that may be added to processed foods. This appendix also lists 55 compounds under the section of nutrients that may be added to processed foods. Upon reviewing this list, several of them could also be considered as non-nutritional substances. These include several carbohydrate derivatives and dietary fibres as well as several miscellaneous substances. Table 2 of this review therefore lists the 16 non-nutritional substances as well as 11 of these "nutrients" in Appendix 1 of the said regulation.

As indicated in Table 2, the list has indicated the food categories to which the non-nutritional substance may be added and the conditions or requirements that must be met for such addition.

Companies may apply to the Indonesian FDA for the use of other non-nutritional substances that are not in the current positive list. Appendix II of the said Regulation has provided an application form that is to be used for such applications. Important data to be provided re the non-nutritional substances include the name of the substance, amount to be added to the food, estimated total daily intake, specifications of the substance, production process, history of use in food and its regulatory status in other countries. Information on the category and name of the food to which the substance is to be added and the purpose of such addition are also required to be provided. Documentation to support the safety of the non-nutritional substance must be provided with the application.

It is to be noted that this Regulation refers to permission for the addition of these non-nutritional substances to foods and does not give permission for making health claims in relation to these food components. Hence, the industry may add these approved non-nutritional substances to processed foods without making any health claim. The Regulation governing health claims for non-nutritional substances is discussion in the next section.

In addition to the non-nutritional substances or food components referred to above, Indonesia Food Regulations also permit the use of yet another category of functional component to foods, namely probiotics and make approved health claims. This is permitted in Regulation 1 of 2022 on Monitoring of Claims on Labels and Advertisements of Processed Foods (FDA Indonesia, 2022). No positive list of probiotics approved for use is published. Industry may apply to the Indonesian FDA for use of microorganisms as probiotics in foods. More details on the provisions for use of probiotics under thie Regulation is given

Table 2. Non-nutritional substances† that may be added to processed foods in Indonesia and the required conditions

No.	*Non-nutritional substance*	*Food category*	*Conditions/Requirements*
1.	Lactic acid	All types of food	Case by case review
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations
2.	Phytostanol, both in the ester and free form	Margarine, margarine spread, dairy products and their processed products, breakfast cereals, mayonnaise and salad dressings, and milk-flavoured drinks	Maximum of 3 g/day
3.	Phytosterol, both in the ester and free form	Margarine, margarine spread, dairy products and their processed products, breakfast cereals, mayonnaise and salad dressings, and milk-flavoured drinks	Maximum of 3 g/day
4.	Glucosamine	All types of food	Maximum of 1500 mg/day
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations
5.	β-hydroxy-β-methylbutyrate (HMB)	All types of food	Case by case review
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations
6.	Isoflavone	All types of food	Case by case review
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations

Table 2. Non-nutritional substances† that may be added to processed foods in Indonesia and the required conditions (cont'd)

No.	*Non-nutritional substance*	*Food category*	*Conditions/Requirements*
7.	Caffeine	All types of food	The requirements refer to the Regulation Number 34 of 2019 concerning Food Categories
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations
8.	Catechin	All types of food	Case by case review
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations
9.	Collagen	All types of food	Sufficient to obtain the desired characteristics
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations
10.	Colostrum	Milk and products	Sufficient to obtain the desired characteristics
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations
11.	Chondroitin	All types of food	Maximum 1200 mg/day
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations
12.	Lutein from *Tagetes erecta*	All types of food	Sufficient to obtain the desired characteristics
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations

Table 2. Non-nutritional substances† that may be added to processed foods in Indonesia and the required conditions (cont'd)

No.	*Non-nutritional substance*	*Food category*	*Conditions/Requirements*
13.	L-Theanine	All types of food	Sufficient to obtain the desired characteristics
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations
14.	Ubiquinone/Coenzyme Q10	All types of food	Case by case review
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations
15.	Zeaxanthin-rich extract	All types of food	Sufficient to obtain the desired characteristics
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations
16.	Lycopene	All types of food	Sufficient to obtain the desired characteristics
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations
17	Isomaltulose	All types of food	In accordance with the provisions of NRV for total carbohydrate
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations

Table 2. Non-nutritional substances† that may be added to processed foods in Indonesia and the required conditions (cont'd)

No.	*Non-nutritional substance*	*Food category*	*Conditions/Requirements*
18	Isomalto-oligosaccharide - IMO	All types of food	Maximum of 30 g/day
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations
19	Sucromalt	All types of food	In accordance with the provisions of NRV for total carbohydrate
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations
20	Beta-glucan	All types of food	In accordance with the provisions of NRV for dietary fibre
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations
21	Fructooligosaccharide – FOS with degree of polymerization 3-10	All types of food	In accordance with the provisions of NRV for dietary fibre
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations
22	Galactooligosaccharide – GOS	All types of food	In accordance with the provisions of NRV for dietary fibre
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations

Table 2. Non-nutritional substances† that may be added to processed foods in Indonesia and the required conditions (cont'd)

No.	*Non-nutritional substance*	*Food category*	*Conditions/Requirements*
23	Inulin	All types of food	In accordance with the provisions of NRV for dietary fibre
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations
24	Resistant maltodextrin/ Resistant starch Type 4/ Distarch Phosphate	All types of food	In accordance with the provisions of NRV for dietary fibre
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations
25	Pectin	All types of food	In accordance with the provisions of NRV for dietary fibre
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations
26	Polydextrose	All types of food	In accordance with the provisions of NRV for dietary fibre
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations
27	Psyllium	All types of food	In accordance with the provisions of NRV for dietary fibre
		Processed food for Special Nutritional Needs in accordance with the provisions of the legislation	Requirements refer to Regulation Number 1 of 2018 concerning Supervision of Processed Food for Special Nutritional Purposes and its amendment regulations

NRV, Nutrient reference value

Source: Appendix I of the Regulation 30 of 2021 concerning Requirements for the Addition of Nutrients and Non-Nutritional Substances to Processed Foods (FDA Indonesia, 2021)

†This table includes 16 non-nutritional substances in Appendix 1 of the said regulation (numbers 1-16), as well as 11 "nutrients" that may be included in this list (numbers 17-27).

in the second broad topic of this review, i.e. on health claims.

Malaysia

The regulatory authority in Malaysia for food safety is Food Safety and Quality Division, Ministry of Health Malaysia (official website: http://fsq.moh.gov.my/).

There is a specific regulation in the Malaysia Food Regulations 1985 (MOH Malaysia, 2017a) which governs the addition of bioactive non-nutritional food components to foods and beverages. This provision is contained in Regulation 26, titled Added Nutrient, which provides for the addition of a variety of nutrients to foods, including "other food components", the term used in this Regulation for bioactive non-nutritional food components. In this Regulation, meaning of the term "added nutrient" has been broadened and is defined as: any mineral, vitamin, amino acid, fatty acid, nucleotide or other food components which, when added singly or in combination to food, improves the nutritional value of the food.

The Regulation further stipulates that only nutrients contained in a positive list in Table I of Twelfth Schedule are permitted to be added to foods (MOH Malaysia, 2017a). This Table lists the following "nutrients" that may be added to foods: 1. Vitamins and minerals, 2. Amino acids, 3. Fatty acids 4. Nucleotides, and 5. Other food components. The last named category, "other food components" have been included as a separate category from the "classical" nutrients of vitamins, minerals, fatty acids and amino acids.

The "other food components" in Table I of the Twelfth Schedule are reproduced in Table 3 of this review. This positive list includes a total of 25 bioactive non-nutritional food components, 11 of which are various forms of dietary fibre. All the food components in this list may be added to foods and beverages, and have arisen from applications from the food industry.

Industry may apply to add "other food components" to the positive list summarised above. Applicants are required to complete a specific form prescribed by FSQD (Application for Addition to Permitted Added Nutrient List) and provide information on the limit of probable intake of the nutrient, the chemical structure, the physiological role of the nutrient, expected beneficial effects, stability and bioavailability, analytical method, data on safety evaluation and approval by other country. The form can be downloaded from this link: http://fsq.moh.gov.my/v6/xs/page.php?id=72.

The scientific information that must be submitted to substantiate the application must be all available literature related to the application including both positive and negative findings on the application. Data from human intervention trials are preferred. Epidemiological and experimental studies and review papers related to the component may be included as supportive evidences. Studies should include those conducted by other organisations or institutions and result of these studies should be published in peer reviewed journals. To facilitate review by expert committee members, summaries of the studies should be presented in table form.

An expert committee has been established to review all applications. The committee consist of relevant practitioners and academicians, particularly those in the field of food, nutrition and health, from related government agencies, academia and professional organisations. The review process for applications is as summarised in the flow chart in Figure 1.

This Regulation refers to permission for the addition of these non-nutritional components to foods and does not give permission for making health claims

Table 3. List of permitted "other food components" that may be added to foods in Malaysia

D-ribose
Calcium 3-hydroxy-3-methyl butyrate monohydrate (CaHMB)/ hydroxy methylbutyrate (HMB) (only permitted in formula dietary food)
Epigallocatechin gallate (EGCG)
Isomaltulose (except in infant formula)
Lactotripeptide [which consists of L-valine-L-proline-L-proline (VPP) and L-isoleucine-L-proline-L-proline (IPP) with proportion of VPP:IPP between 0.56 to 1.77 (addition is only permitted for fruit juice, vegetable juice and milk product except for infant formula, follow-up formula and formulated milk powder for children)]
Mixture containing 50 percent (weight per weight) galactooligosaccharide (GOS) and 50 per cent (weight per weight) polydextrose (PDX)
Sialic acid (from milk)
Plant sterols or plant stanols or phytosterols or phytostanols (comprising mainly of sitosterol, campesterol, stigmasterol and other related plant stanol)
Plant sterol esters (comprising mainly of campesterol ester, stigmasterol ester and beta-sitosterol ester)
Soy protein
Sucromalt (only permitted in formula dietary food)
Beta glucan from yeast
Bovine lactoferrin
Slowly Digestable Starch (SDS)
Dietary fibre
Acacia gum/gum arabic (only from Acacia senegal and Acacia seyal)
Galactooligosaccharide (GOS)
High amylose maize resistant starch (HAMRS) (not permitted in infant formula and follow-up formula)
Inulin
Beta glucan from oat soluble fibre
Beta glucan from barley
Oligofructose/fructooligosaccharide
Oligofructose-inulin mixture containing shorter chain inulin (oligofructose DP 3-9) and longer chain inulin (inulin DP ≥ 10) in a 50:50 ratio ± 10% each
Oligosaccharide mixture containing 90 per cent (weight per weight) of oligogalactosyl-lactose [galactooligosaccharides (GOS)] and 10 per cent (weight per weight) oligofructosyl saccharose [long chain fructooligosaccharide (lcFOS)]
Polydextrose
Resistant dextrin/resistant maltodextrin (not permitted in infant formula and follow-up formula)

Source: Table I of the Twelfth Schedule, Malaysia Food Regulations 1985 (MOH Malaysia, 2020)

in relation to these food components. Hence, the industry may add these approved components to foods and beverages without making any health claim. The regulation governing health claims for bioactive non-nutritional food components is discussed in the next section.

In addition to the food components referred to above, Malaysia Food Regulations also permit the addition of yet another category of functional component to foods, namely probiotics. This is permitted under Regulation 26A which spells out details such as definition of probiotics, conditions required namely that the probiotic cultures shall remain viable and the probiotic count shall not be less than 10^6cfu/ml or cfu/g during the shelf life of the food containing these, and labelling requirements. The regulation also includes a positive list of 32 permitted strains of probiotics that may be used. Industry may apply for use of new probiotic strains using a prescribed form which spells out in detail the required information to be submitted for consideration by FSQD (MOH Malaysia, 2017b; Tee, Hardin & Au, 2021).

Myanmar

Governing authority on food safety in Myanmar would mainly be Department of Food and Drug Administration (FDA), Ministry of Health and Sports (official website: https://www.mohs.gov.mm).

There is no specific regulation regarding the use of bioactive non-nutritional food components in Myanmar. No positive list has been established for these food components that have been approved for use. FDA can inform industry and consumers if there are enquiries if an ingredient of interest is permitted to be used in foods or beverages.

Companies may apply for the use of bioactive non-nutritional food components and be considered by the FDA on a case-by-case basis. Bioactive components that may be considered are ingredients of known food sources such as plant/botanical, animal and microbial including their metabolites, derivatives or enzymes. Scientific evidence on its health benefits and safety must be submitted for consideration. For very new (novel) ingredient/bioactive substance or extract from uncommon food source, FDA evaluates the scientific evidence relevant to its specific health benefits as a preliminary assessment (document review only).

There is however no specific framework established for reviewing applications for use of new bioactive non-nutritional food components, and no independent scientific or expert committee has been established yet for the critical review.

There does not appear to be a specific regulation on probiotics in Myanmar, though products containing these microorganisms are available on the market (Koirala & Anal, 2021).

Philippines

The regulatory authority in the Philippines on food safety is the Food and Drug Administration (official website: https://www.fda.gov.ph/).

There is no specific regulation for addition of bioactive non-nutritional food components to foods and beverages. However, bioactive food ingredients may be permitted to be added to foods and dietary supplements. In the definition of food and food/dietary supplement based on Republic Act 9711 (Republic of the Philippines, 2009), “any substances” and “dietary ingredients” may be added:

“Food means any processed substance, which is intended for human consumption and includes drinks for human beings, beverages, chewing gum and “any substances”, which have been used as an ingredient in the manufacture, preparation or treatment of food.”

“Food/dietary supplement’ means a processed food product intended

Figure 1. Flow chart of amendment of food regulations under Food Act 1983

to supplement the diet that bears or contains one or more of the following dietary ingredients: vitamin, mineral, herb, or other botanical, amino acid, and dietary substance to increase the total daily intake".

Bioactive substances like flavonoids, carotenoids, antioxidants, phytochemicals, lycopene, fatty acids, peptides are collectively termed as "any substance".

There is no positive list of bioactive non-nutritional food components permitted for food use. FDA can inform stakeholders through replies to their inquiries via letters, emails, calls or face-

to-face discussion (for walk-in clients) and through results of their registration application.

Industry may apply for the use of bioactive non-nutritional food components as provided for in Administrative Order No. 2014-0029 Rules and Regulations on the Licensing of Food Establishments and Registration of Processed Food, and Other Food Products, and For Other Purposes (DOH Philippines, 2014). Bioactive non-nutritional food components are approved by product registration provided that safety is established. The applicant may file an online application which will be assessed by Center for Food Regulation and Research based on the submitted safety data, if any, and other documentary requirements based on this A.O. 2014-0029. There is no specific prescribed form for submitting applications. No details of the requirements for application or type of scientific information required have been provided.

Besides the above mentioned bioactive non-nutritional food components, Philippines also permit the addition of probiotics to foods. The Bureau of Food and Drug (BFAD) Circular No. 16s 2004 has provided a definition of probiotics, specific labelling requirements, and published a list of 5 genera/species of microorganisms to be used as probiotics in the Philippines (BFAD, 2004). Companies may apply for the use of probiotics not in the current approved list using the electronic registration system under FDA Circular 2016-014 (FDA Philippines, 2016). Scientific data must be submitted to demonstrate that the new bacterial strain must provide evidence of safety and effectiveness as a probiotic.

Singapore

The Singapore Food Agency (SFA) is the authority to oversee food safety and food security from farm-to-fork in Singapore (official website: www.sfa.gov.sg).

Bioactive non-nutritional food components are permitted to be added to foods and beverages. However, there is no specific regulation for this purpose. In most cases, the use of bioactive food components is tied up with other function claims, which are permitted by SFA. The regulation governing health claims for such bioactive food components is discussed in the next section. SFA also permits bioactive food components which do not have pre-approved function claims to be used in foods. SFA does not publish a positive list of permitted bioactive food components.

On the other hand, with the increased interest on the production of novel foods/ingredients which do not have a history of being consumed by humans as food, SFA has released a document on the food safety information that would be required for novel food safety assessment which was last updated in September 2022 (SFA, 2022). The information requested include potential food safety risks such as toxicity, allergenicity, safety of its production method, and dietary exposure arising from consumption. Companies that wish to submit the application must also provide detailed information on the materials used in their manufacturing processes and how these manufacturing processes are controlled to prevent food safety risks. These safety assessments will be reviewed and updated periodically to facilitate the safety assessments by the industry and ensure food safety.

In terms of probiotics, unlike in Indonesia, Malaysia, Philippines and Thailand, there is no specific regulation regarding the use of probiotics in foods in Singapore. There is also no published list of permitted strains of probiotics that may be used. Nevertheless, Singapore Food Regulations permits strains of *bifidobacteria* and *lactobacillus* that have a proven long history of safe use to be used as probiotics in suitable categories of food products, including cultured milk drink and yoghurt (SFA, 2021).

For the use of new microorganisms for use as probiotics in foods in Singapore, an application may be submitted to SFA. All needed documents must be submitted, including data on identification, characterisation and safety for use by the intended target groups (Tee *et al.*, 2021).

Thailand

Thailand Food and Drug Administration (Thai FDA), Ministry of Public Health is the regulatory authority for food safety in Thailand (official website: www.fda.moph.go.th).

There is no specific regulation, definition or description of bioactive non-nutritional food components in the food regulations in Thailand. Nevertheless, these components are permitted to be added to foods and beverages with pre-marketing approval.

The uses of bioactive non-nutritional food components are tied up with other function claims, which are permitted by Thai FDA. Industry may apply for the use of a food component and the associated other function claim on a case-by-case basis. This is summarised in the section on regulatory framework on health claims related to bioactive non-nutritional food components.

Applications for use of bioactive non-nutritional food components is linked to applications for other function claims. If the food component is a novel ingredient, safety assessment of the component is needed. Refer to section below on regulatory framework for health claims.

Besides the above provisions, Thai FDA also permits the use of probiotics in foods, according to the following notifications of Ministry of Public Health (MoPH):

a. Notification of the Ministry of Public Health (No. 339) B.E. 2554 (2011) Re: Use of Probiotic Microorganisms in Foods (MoPH Thailand, 2011).
b. Notification of the Ministry of Public Health (No. 346) B.E 2555(2012) Re: Use of Probiotic Microorganisms in Foods (No.2) (MoPH Thailand, 2012).

The MoPH notification No. 339 provides details of the probiotics, including definition, number of viable microorganisms required in the product, labelling requirements and has provided a list of 23 species of microorganisms that are permitted for use in foods. Manufacturers or importers in Thailand may apply for the use of probiotic microorganisms other than those specified in the positive list. Applications can be submitted using a form online and provide the needed information, including scientific substantiation on the safety and functional role or health benefits (MoPH Thailand, 2011).

Vietnam

The authority responsible for management of food safety issues of domestic and imported foods in Vietnam is the Vietnam Food Administration (VFA), of the Ministry of Health (official website: www.vfa.gov.vn).

There is no specific regulation for the use of bioactive non-nutritional food components in Vietnam. Nevertheless, ingredients with biological activities may be added to specific functional foods. This is provided for in the Ministry of Health Vietnam circular on regulating functional foods (MOH Vietnam, 2014). In this Circular, functional foods include supplemented foods, health protection foods (health supplement, food supplement, dietary supplement), medical foods and foods used for special dietary uses.

In Article 2 of this MOH 2014 circular, supplemented foods have been defined as conventional foods supplemented with micronutrients and other healthful components for the body such as vitamins, minerals, amino acids, fatty acids, enzymes, probiotics, prebiotics and other biologically active substances.

Table 4. Summary of regulatory framework for health claims related to bioactive non-nutritional food components in eight SEA countries

	Permitted health claims†	*Regulation or guideline*	*Positive list of permitted health claims*	*Application for new health claims*
Codex Alimentarius	Other function claim, reduction of disease risk claim	Codex Guidelines for Use of Nutrition and Health Claims CAC/GL 23-1997	Not provided	Not applicable
Brunei Darussalam	Other function claim, reduction of disease risk claim	No specific regulation; Codex Guidelines are applicable	No positive list of health claims and those related to probiotics	Yes; no specific framework for applications, applications will be reviewed on a case-by case basis
Indonesia	Function claims for nutrients and non-nutritional substances, reduction of disease risk claim	Regulation 1/2022, Republic of Indonesia	List provided in regulation; 10 function claims for 5 groups of non-nutritional substances. Only one generic function claim permitted for probiotics	Applications can be made using a prescribed application form. Regulatory framework established to review applications, procedure for assessment of applications summarised in regulation. Require scientific data to substantiate intended claim.
Malaysia	Other function claim permitted. Reduction of disease reduction claim not permitted	Regulation 18F (amendment 2020) of Malaysia Food Regulations 1985	List provided in regulation for 22 non-nutritional components for 43 other function claims. Only one generic function claim permitted for probiotics	Applications for other function claims can be made using a prescribed application form. Regulatory framework established to review applications, require scientific data to substantiate intended claim
Myanmar	Other function claim, reduction of disease risk claim	No specific regulation	No positive list of health claims and those related to probiotics	No specific framework for review, require scientific substantiation

Table 4. Summary of regulatory framework for health claims related to bioactive non-nutritional food components in eight SEA countries (cont'd)

	Permitted health claims[†]	*Regulation or guideline*	*Positive list of permitted health claims*	*Application for new health claims*
Philippines	Other function claim, reduction of disease risk claim	Bureau Circular No. 2007-002, Republic of Philippines	No list of other function claims provided, but four pre-approved claims related to probiotics available	Applications for health claims may be made under Administrative Order 2014-0029; no prescribed form. Companies may apply for use of health claims on probiotics
Singapore	Other function claim, reduction of disease risk claim	Guide to Food Labelling and Advertisements, 2021, Singapore Food Agency	A list of 17 other function claims for 12 bioactive non-nutritional food components provided. List includes four function claims related to probiotics	Applications for health claims can be made using a prescribed application form. Regulatory framework established to review applications, require scientific data to substantiate intended claim
Thailand	Other function claim, reduction of disease risk claim	Provided under Food Act B.E. 2522. Regulation on health claims is being drafted. Permitted health claims and associated conditions are given in the Public Manual on Requesting for assessment of health claim by Thai FDA 2020	No list of other function claims provided. One generic pre-approved claim related to probiotics available	The Public Manual provides details of regulatory framework, requirements for application and review of process; require scientific substantiation. Industry may apply for health claims on probiotics, with scientific substantiation
Vietnam	Other function claim, reduction of disease risk claim	No specific regulation; MOH Circular on Regulating Management of Functional Foods makes provision for health claims for functional foods	No positive list of health claims and those related to probiotics	Industry may apply for health claims with scientific substantiation. No review framework established

[†]Nutrition function claims are not included as they relate to physiological role of nutrients whereas this review focuses on other function claim and reduction of disease risk claim which refer to the potential health effects of non-nutritional functional food components

Currently, there is no published list of "healthful components for the body" that are permitted to be used in supplemented foods. There is no specific framework, but companies may apply to the MOH Vietnam for the use of any food components/constituents that they intend to add to their supplemented food products.

In Vietnam, there is no specific regulation or legal definition of probiotics. However, as indicated above, the MOH Circular allows the addition of "healthful components for the body" to supplemented foods, and probiotics are included as one of these substances. The sale of probiotics in foods is regulated through several government agencies, including the Vietnam Food Administration (VFA) and inspectors of the Ministry of Health, Department of Health and Food Safety Management Agencies, Vietnam Directorate of Market Surveillance (Ministry of Industry and Trade) and its agencies at the provincial levels (Tee *et al.*, 2021).

Manufacturers may apply to the VFA to register a bacteria strain for use under supplemented food. Permission for the use of the bacteria strain is given based on the strength of scientific evidence of the product or ingredient. More details on the management of probiotics under functional foods, which includes supplemented foods and dietary supplements is provided in Circular No. 43/2014/TT-BYT (MOH Vietnam, 2014).

Status of regulatory framework for health claims related to bioactive non-nutritional food components

Table 4 summarises the status of health claims permitted to be made in relation to bioactive non-nutritional food components in Codex framework and the eight SEA countries in this review. As can be seen from the Table, all the countries in the review permit the use of other function claim and reduction of disease risk claim and the definitions of these are line line to those of Codex guideline. However, the regulatory framework in each country varies considerably, details of which are summarised in the following paragraphs.

Codex Alimentarius

As summarised in the first section of the results of the review, Codex Guidelines for Use of Nutrition and Health Claims CAC/GL 23-1997 has made provisions for making of health claims (FAO/WHO, 2013). In the context of this review, the relevant health claims are other function claim and reduction of disease risk claim, linking the role of "consumption of foods or their constituents" and health. As previously explained, the food constituents in these Codex health claims refer to non-nutritional food components that are bioactive and may bring about positive health effects.

Brunei Darussalam

The Public Health (Food) Regulation (R1, chapter 182) of Brunei Darussalam has not provided specific regulations on health claims (MOH Brunei, 2001). The Regulation has stipulated that under such circumstances, Codex Alimentarius standards shall apply, including the Codex guidelines on claims CAC/GL 1-1979 (FAO/WHO, 2009), and the Codex guidelines for use of Nutrition and Health Claims CAC/GL 23-1997 (FAO/WHO, 2013).

There is no positive list of permitted health claims. The industry may enquire to the BDFA, through letters, emails or other channels, whether a particular health claim is permitted. Claims that are intended to be included on the packaging or to be displayed during sale should first be submitted to the BDFA and shall be reviewed on a case-by-case basis.

There is also no specific framework for application for use of health claims for bioactive non-nutritional food components. Nevertheless, companies

may apply to the BDFA for consideration on a case-by-case basis by experts appointed by the Authority.

Indonesia

In Indonesia, claims on labels of processed foods are provided for in Regulation 1 of 2022 on Monitoring of Claims on Labels and Advertisements of Processed Foods (FDA Indonesia, 2022). The permitted claims on food labels as stipulated in Article 2 of the Regulation are: claims on nutrients and non-nutritional substances, health claims, isotonic claim, vegan claim and claims related to microorganisms. Non-nutritional substances in these regulations refer to compounds or bioactive/functional components contained in food that do not function as nutrients but affect health. This is the same as defined in Regulation 30 of 2021 on Requirements for the Addition of Nutrients and Non-nutritional Substances to Processed Foods (FDA Indonesia, 2021).

Health claims in Regulation 1 of 2022 are *"any form of description stating, suggesting or implying that there is a relationship between food or food ingredients and health"*. Three types of health claims are permitted in these regulations: (a) function claim for nutrients and non-nutritional substances, (b) reduction of disease risk claim and (c) glycaemic claim. Function claims for nutrients and non-nutritional substances are *"claims that describe the physiological role of nutrients/non-nutritional substances for normal growth, development and function of the body"*. The reduction of disease risk claim is a *"claim that links food consumption or food components in the total diet with a reduced risk of developing a disease or certain health conditions"*.

This definition of health claim is similar to that of Codex Guidelines for Use of Nutrition and Health Claims CAC/GL 23-1997 (FAO/WHO, 2013). However, it should be pointed out that the classification of health claims in these regulations are not the same as those in the Codex Guidelines, as outlined above. It is noted that the term "other function claim" is not used and the term function claim is used for both nutrients and non-nutritional substances.

Annex VII of Regulation 1 of 2022 has provided a list of the permitted function claims for nutrients and non-nutrients (FDA Indonesia, 2022). The permitted function claims for non-nutrients are extracted and presented in Table 5 of this review. These include ten function claims for five non-nutritional substances, namely several types of dietary fibre (which includes psyllium, beta glucan from oats and/or barley, inulin from chicory and pectin from fruits; resistant maltodextrin/resistant dextrin, insoluble dietary fibre and slowly digestible starch), isomaltulose, sucromalt, xylitol and phytosterols/phytostanols and their esters. There are various conditions to be met to be eligble to make these claims.

This Regulation also permits making of reduction of disease risk claim. However, there is no published positive list of permitted claims. It is interesting to note that under the list of nutrient function claims in Appendix VII, one of the permitted claims for folic acid is:

Supplemental intake of folic acid improves maternal folate status; low maternal folate status is a risk factor for development of neural tube defect in the developing fetus.

As indicated above, permitted claims under Article 2 of Regulation 1/2022 include claims related to microorganisms, with the following conditions:

a. processed foods using live microorganisms must meet the requirements of safety, quality, and benefits;
b. types of microorganisms that can be used on processed food is determined by the Head of the Agency;

Table 5. List of permitted function claims for non-nutritional substances in Indonesia

Non-nutritional substance	*Claim statement*
Dietary fibre†	1. Soluble dietary fibre (psyllium, beta glucan from oats and/or barley, inulin from chicory and pectin from fruits) can help protect/maintain the function of the digestive tract Conditions: a. Processed food must include fibre as a constituent; and b. Processed food contains at least 3 g per serving of soluble dietary fibre
	2. Soluble dietary fibre (resistant maltodextrin/resistant dextrin) can help protect/maintain the function of the digestive tract Conditions: a. Processed food must include fibre as a constituent; and b. Processed food contains at least 5 g per serving of soluble dietary fibre
	3. Soluble dietary fibre (psyllium, beta glucan from oats and/or barley, inulin from chicory and pectin from fruits) can help lower blood cholesterol levels when accompanied with a diet low in saturated fat and cholesterol Conditions: a. Processed food must include fibre as a constituent; b. Processed food contains at least 3 g per serving of soluble dietary fibre c. Maximum total fat is 3 g per serving; or if the serving is less than 50 g, maximum total fat content is 3 g per 50 g; d. Maximum saturated fat is 1 g per serving and maximum calories from saturated fat is 15%; if the amount per serving is less than 100 g, maximum saturated fat content is at 1 g per 100 g and maximum calories from saturated fat 10%; and e. Maximum cholesterol at is 20 mg per serving; or if the serving is less than 50 g, maximum cholesterol content is 20 mg per 50 g Warning: The claim must be accompanied by the statements: a. Food consumption should be accompanied by the consumption of food low in fat, low in saturated fat and/or low cholesterol b. Consumption of these products must be accompanied by a healthy lifestyle
	4. Soluble dietary fibre (psyllium, beta glucan from oats and/or barley, inulin from chicory and pectin from fruits) and resistant maltodextrin/resistant dextrin can contribute to lowering the rise of blood sugar after a meal if accompanied with a balanced diet Conditions: a. Processed food must include fibre as a constituent; and b. Processed food contains at least 3 g per serving of soluble dietary fibre c. If the source of soluble dietary fibre used is in the form of resistant maltodextrin/resistant dextrin, then the amount of soluble dietary fibre should be at least 5 g per serving

Table 5. List of permitted function claims for non-nutritional substances in Indonesia (cont'd)

Non-nutritional substance	*Claim statement*
	5. Insoluble dietary fibre facilitates bowel movements (laxative), when accompanied by drinking enough water. Conditions: a. Processed food must include fibre as a constituent; and b. Processed food contains at least 3 g per serving of insoluble dietary fibre
	6. Contains slowly digestible starch (SDS) .....% of total starch. Conditions: a. At least 55% of energy is from available carbohydrates; b. At least 55% of available carbohydrates is total starch; and c. At least 40% of total starch is slowly digestible starch (SDS).
Isomaltulose	Isomaltulose is a sugar substitute sweetener that does not cause a rapid rise in blood glucose after consuming this product Condition: The processed food must meet the requirements for low sugar claim
Sucromalt	Sucromalt is a sugar substitute sweetener that does not cause a rapid rise in blood glucose after consumption of this product Conditions: a. The processed food must meet the requirements for low sugar claim b. The composition of sucromalt consists of fructose (35-45% dry weight), leucrose (7-15% dry weight), mono- and disaccharides (at most 3% dry weight) and oligasaccharides (40-60% dry weight).
Xylitol	Helps maintain naturally white teeth Conditions: a. The processed food contains at least 15% xylitol; b. The processed food must also contain at least 0.5% calcium carbonate; and c. Inclusion of the claim must be accompanied by inclusion of advice to consumers to keep brushing their teeth regularly
Phytosterol/ phytostanol, both in the ester and free form	Phytosterol/phytostanol/physterol ester/phytostanol ester helps lower cholesterol in patients with hyperlipidemia / hypercholesterolemia, when accompanied by a diet low in saturated fat and cholesterol Conditions: a. The processed food must contains 1.5 – 3 g of phytosterols/ phytostanol per day; and b. The claim may only be declared on margarine, spreadable margarine, dairy products and their processed products, breakfast cereals, mayonnaise, salad dressings and milk-flavoured drinks

Source: Annex VII of Regulation 1/2022 of the Indonesian FDA (2022)
[†]Must meet the requirement for claim as "source"

c. claims related to microorganisms can only be used after obtaining written approval from the Head the Agency. There is no published list of permitted health claims on microorganisms.

In relation to making health claims, Regulation 1/2022 has pointed that the following must be taken into consideration:

a. type, quantity, and function of nutrients or non-nutritional substances;
b. a reasonable amount of food consumed per day;
c. balanced food consumption pattern;
d. public health condition;
e. the appropriateness of the food to serve as a carrier for the nutrient or non-nutritional substance; and
f. appropriateness of the food to include claims.

In addition, Article 5 of the said Regulation summarises the requirements for making claims on labels of processed foods. Besides meeting the basic requirements for the relevant food category, processed foods must meet the following nutritional criteria before a claim (including other function claim) can be placed on the label:

One serving of the processed food must not exceed the following nutritional parameters:

- 18 g total fat;
- 6 g of saturated fat;
- 60 mg cholesterol; and
- 300 mg sodium.

This regulation has also clearly specified that manufacturers are prohibited from the following:

a. include claims for processed foods intended for infants, except otherwise provided for;
b. include claims for reducing disease risk for processed foods intended for children aged 1 (one) to 3 (three) years, except otherwise provided for;
c. include claims declaring free of nutritional/non-nutritional substances in processed foods which naturally do not contain nutritional/non-nutritional substances, except otherwise provided for;
d. contains a statement that the consumption of the processed food can meet all the nutritional needs;
e. list claims that leverage concerns of consumers;
f. include claims that cause consumers to consume the processed food in an inappropriate manner; and/or
g. include a claim that illustrates that the processed food can prevent, treat or cure disease.

Industry may apply for additional health claims not currently in the positive list via an online system of the Indonesian FDA. Chapter III of the said Regulation 1/2022 deals with review of applications for new claims (FDA Indonesia, 2022). The information that are required to be submitted for the intended claim is provided in Annex XI of the said regulations which has six sections. Part A stipulates the requirements for claims which are not related to microorganisms (i.e. claims related to nutritional and non-nutritional substances and health claims, isotonic claim and vegan claim). Information required include those related to the intended claim and regarding the foods bearing the claim, as well as scientific substiation documents.

Parts B-F of this Annex XI provide the requirements for claims related to microorganisms. Parts B and C lists the data requirements for starter cultures with identified and unidentified microorganisms in fermentation foods; part D presents data requirements for microorganisms for use as

food ingredients (without claim as probiotic). Part E lists in detail the data requirements for use of probiotics in processed foods. These include full details of the microorganism, functional characteristics, safety data and beneficial effects. Details on the intended food for addition of the probiotic are also required, including amount of the microorganism to be added.

For microorganisms which have been approved for use in foods and beverages, industry may apply for functional health claims or reduction of disease risk claims. Details of the requirements are provide in Part F of Annex XI. The requirements for the data to be submitted to substantiate the intended claim are indicated in this part.

Regulation 1/2022 also details out the procedure for review of applications for claims in Annex XII. Detailed information on the food must be provided including physical and chemical properties of the food, metabolism, toxicological data from animals as well as tolerance studies on humans. For reduction of disease risk claim, evidence must preferably be provided through randomised controlled trials. A list of items to be noted when conducting human trials are provided in the Annex, including subjects of the study, dietary data and statistical methods. The appropriate biomarkers and end point are also explained. Applications shall be evaluated by an independent panel of experts. The scientific findings submitted should demonstate that consumption of an amount of the food as recommended shall bring about a statistically and clinically significant claimed effect.

Annex XIII outlines the procedure for review of the safety and beneficial effects of microorganisms in foods, either in fermentation process or added to food with or without the inclusion of probiotic claims and/or health claims. Of particular relevance to this publication are the sections related to the review of probiotics either with or without health claims. The permitted claim on the label of approved products are:

- claim of "Probiotics" which can be accompanied by a generic claim, *"Helps maintain a healthy digestive tract"*.
- health claims (function claims and disease risk reduction claims) can be listed according to the findings of beneficial effects obtained.

All the types of claims pemitted under this Regulation must be proven by the results of the analysis of accredited laboratories or government laboratories. In the case of imported processed foods, analysis results can be issued by:

a. laboratory from the country of origin that has been accredited by the competent authority in the country of origin; or
b. laboratory from the country of origin that has mutual recognition agreements with authorised institutions and/or accredited laboratories in Indonesia.

Malaysia

Health claims permitted in the Malaysia Food Regulations 1985 are nutrient function claim and other function claim. Disease risk reduction claim is not permitted in Malaysia. Relevant to this review is other function claim in Regulation 18F (MOH Malaysia, 2020), defined as:

> *a claim that describes specific beneficial effect of other food component in the food that gives positive contribution to health or improvement of a function of the body.*

The said Regulation has provided a positive list of permitted other function claims as given in Table IV to the Fifth A Schedule (see Table 6). As listed in the Table, there are 22 bioactive non-nutritional food components in

this positive list for 43 other function claims. Each other function claim in the Table also lists the minimum amount of the bioactive food component and other conditions that must be met. The Regulation also emphasises that other function claim shall not imply or include any statement to the effect that the nutrient would afford a cure or treatment for a disease or protection from a disease.

The industry may apply for new other function claims not in the positive list. Applicants must be made in a specific form prescribed by FSQD (Application for Nutrition Function Claim) (available from MOH website: http://fsq.moh.gov.my/v6/xs/page.php?id=72). A prerequisite is that the bioactive non-nutritional food components must first be approved for use as a permitted added nutrient in Table I of the Twelfth Schedule. The items required in the application form are similar to that for applying for addition of bioactive food components to the positive list. Besides the basic information required, the application must provide scientific data to substantiate the other function claim and state the minimum amount required for the claimed effect as specified in Table III to the Fifth A Schedule. More details of the data required and the application review process by the expert committee are as summarised in the section on applying for use of new bioactive non-nutritional food components.

As discussed in the previous section, Regulation 26A of Malaysia Food Regulations 1985 permits the addition of probiotic cultures to foods (MOH Malaysia, 2017b). With regard to permitted health claim for probiotic-containing foods, only a pre-approved generic function claim is permitted under this regulation, namely: *"Probiotic cultures help in improving intestinal or gut function"* or any other words of similar meaning. Malaysia does not allow disease risk reduction health claims to be made on foods, including probiotic-containing foods, although 'other function claims' may be considered, if supported by scientific substantiation.

Myanmar

There is no specific regulatory framework for reviewing applications for health claims related to bioactive non-nutritional food components. Applications may be submitted to FDA, accompanied by scientific substantiation, and will be reviewed and permitted on a case-by-case basis. FDA does not publish a positive list of permitted health claims. FDA can inform industry and consumers if there are enquiries if a particular health claim is permitted. It is however emphasised that the permitted claim should not state or imply that it can prevent, treat or cure any disease. There are no provisions for health claims related to probiotics.

Philippines

Health claims are permitted by the FDA in the Philippines, via Bureau Circular No. 2007-002, Guidelines in the Use of Nutrition and Health Claims in Food (FDA Philippines, 2007). This circular announces the adoption of the Codex Alimentarius Commission Guidelines for Use of Nutrition and Health Claims (CAC/GL 23-1997) (FAO/WHO, 2013) in the evaluation of the use of nutrition and health claims in food labelling and in the advertisement of food products. As summarised above, these Codex guidelines provide for making of other function claims for beneficial effects related to bioactive non-nutritional food components.

FDA of the Philippines does not publish a positive list of permitted health claims, including those related to bioactive non-nutritional food components. Stakeholders can be informed of permitted health claims through replies to their inquiries via letters, emails, calls or face-to-face

Table 6. List of permitted other function claims and required conditions in Malaysia

Component	*Claims*	*Minimum amount required*	*Conditions*
Beta glucan	Beta glucan from (state the source) helps reduce cholesterol.	0.75 g per serving	(i) Source of beta glucan shall be from oat and barley. (ii) The food to be added with beta glucan shall also contain total dietary fibre for not less than amount required to claim as "source": – 3 g per 100 g (solids) – 1.5 g per 100 ml (liquids) (iii) There shall be written on the label the following statement: "Amount recommended for cholesterol lowering effect is 3 g per day".
Beta glucan from barley soluble fibre	(i) Beta glucan from barley soluble fibre helps lower the rise of blood glucose provided it is not consumed together with other food. (ii) Beta glucan from barley soluble fibre contributes to the reduction of the rise in blood glucose provided it is not consumed together with other food.	6.5 g per 100g	(i) This claim is only permitted in cereal and cereal based product. (ii) This claim is only permitted for product where the macronutrient profile (carbohydrate, protein and fat) complies with Recommended Nutrient Intake (RNI) Malaysia. (iii) There shall be written on the label the following statement: "Before deciding to use this product, seek the advice of a health professional".
Beta glucan from oat soluble fibre	Beta glucan from oat soluble fibre helps to lower the rise of blood glucose provided it is not consumed together with other food.	6.5 g per 100 g	(i) This claim is only permitted in cereal and cereal based product. (ii) This claim is only permitted for product where the macronutrient profile (carbohydrates, proteins and fats) complies with the Recommended Nutrient Intake (RNI) Malaysia. (iii) There shall be written on the label of cereal and cereal based product the following statement: "Before deciding to use this product seek the advice of a health professional".
Beta glucan from yeast	Beta glucan from yeast may help to support immune system associated with colds.	0.05 g per serving	(i) Beta glucan from yeast shall be more than 75% on a dry weight basis. (ii) There shall be written on the label the following statement: "Amount recommended for claim effect is 0.2 g per day".

Table 6. List of permitted other function claims and required conditions in Malaysia (cont'd)

Component	*Claims*	*Minimum amount required*	*Conditions*
Beta palmitin	(i) Beta palmitin contributes to increase calcium absorption. (ii) Beta palmitin contributes to increase fat absorption.	(i) >18 percent C16:0 content based on total fatty acids (ii) > 40 per cent C16:0 in sn-2 position based on total C16:0 content	Nil
Bifidobacterium lactis	(i) *Bifidobacterium lactis* helps to improve a beneficial intestinal microflora. (ii) *Bifidobacterium lactis* helps to reduce the incidence of diarrhea.	1 x 10^6 minimum viable cells per gram	These claims are only permitted in infant formula, follow-up formula, formulated milk powder for children and cereal based food for infant and children.
Calcium 3-hydroxy-3-methyl butyrate monohydrate (CaHMB)	(i) CaHMB helps to regain strength. (ii) CaHMB supports tissue building.	1.5 g per serving	This claim is only permitted in formula dietary foods.
Galacto-oligosaccharide (GOS) and polydextrose (PDX) mixture	GOS and PDX mixture is a prebiotic. GOS and PDX mixture is a bifidogenic.	0.4g per100ml (0.2g per100ml GOS and 0.2 g per 100ml PDX)	(i) Mixture containing 50 per cent (weight over weight) GOS and 50 percent (weight over weight) PDX. (ii) These claims are only permitted in infant formula and follow-up formula.
Oligofructose-inulin mixture	Oligofructose-inulin mixture helps to increase calcium absorption and increase bone mineral density when taken with calcium rich food.	2 g per serving	(i) Oligofructose-inulin mixture containing shorter chain inulin-(oligofructose DP 3-9) and longer chain inulin (inulin DP ≥10) in a 50:50 ratio ± 10% each. (ii) Total fructant content in the mixture shall be more than 90 per cent on dry weight basis.

Table 6. List of permitted other function claims and required conditions in Malaysia (cont'd)

Component	*Claims*	*Minimum amount required*	*Conditions*
Oligosaccharide mixture containing galacto-oligosaccharide (GOS) and long chain fructo-oligosaccharide (lcFOS)	Oligosaccharide mixture containing GOS and lcFOS helps to improve the gut or intestinal immune system of infant.	The component (oligosaccharide mixture) shall be 0.8 g per 100 ml.	(i) Oligosaccharide mixture containing 90 per cent (weight per weight) GOS and 10 per cent (weight per weight) lcFOS. (ii) This claim is only permitted in infant formula and follow up formula.
	(i) Oligosaccharide mixture containing GOS and lcFOS is a prebiotic. (ii) Oligosaccharide mixture containing GOS and lcFOS is a bifidogenic. (iii) Oligosaccharide mixture containing GOS and lcFOS helps to increase intestinal bifidobacteria. (iv) Oligosaccharide mixture containing GOS and lcFOS helps to maintain a good intestinal environment.	0.4 g per 100 ml	(i) Oligosaccharide mixture containing 90 per cent (weight per weight) GOS and 10 per cent (weight per weight) lcFOS. (ii) These claims are only permitted in infant formula, follow up formula and formulated milk powder for children. (iii) The component (oligosaccharide mixture) shall not exceed 0.8 g per 100 ml.
Resistant dextrin or resistant maltodextrin	Resistant dextrin or resistant maltodextrin is a soluble dietary fibre that helps to regulate or promote regular bowel movement.	2.5 g per serving	Addition and claim for resistant dextrin or resistant maltodextrin are not permitted in infant formula.
	(i) Resistant dextrin or resistant maltodextrin is a prebiotic. (ii) Resistant dextrin or resistant maltodextrin is a bifidogenic. (iii) Resistant dextrin or resistant maltodextrin helps increase intestinal bifidobacteria. (iv) Resistant dextrin or resistant maltodextrin helps maintain a good intestinal environment.	4 g per serving	The minimum amount that must be present in the food to give the claim effect is proposed to be 8 g per day.

Table 6. List of permitted other function claims and required conditions in Malaysia (cont'd)

Component	*Claims*	*Minimum amount required*	*Conditions*
Docosahexaenoic acid (DHA) and Arachidonic acid (ARA)	DHA and ARA helps to contribute in the visual development of infant.	A combination of 17 mg per 100 kcal DHA and 34 mg per 100 kcal of ARA	This claim is only permitted in infant formula product.
D-ribose	D-ribose helps to promote energy recovery during or after physical activities.	3 g per serving	i) This claim is only permitted in formula dietary foods. ii) There shall be written on the label the following statement: "Do not exceed 2 servings per day".
Inulin	(i) Inulin is a prebiotic. (ii) Inulin is a bifidogenic. (iii) Inulin helps to increase intestinal bifidobacteria and maintain a good intestinal environment.	1.25 g per serving	This minimum level is specified for food other than infant formula.
		0.4 g per 100 ml on a ready to drink basis	(i) This minimum level is specified for infant formula only. (ii) The component (inulin and oligofructose/ fructooligosaccride (FOS)) shall not exceed 0.6 g per 100 ml.
Isomaltulose	(i) Isomaltulose is a slowly hydrolysed to glucose and fructose compared to sucrose. (ii) Isomaltulose provides longer lasting energy compared to sucrose. (iii) Isomaltulose is a slowly released source of energy compared to sucrose.	15 g per serving	Addition and claim for isomaltulose are not permitted in infant formula.

Table 6. List of permitted other function claims and required conditions in Malaysia (cont'd)

Component	*Claims*	*Minimum amount required*	*Conditions*
High amylose maize resistant starch (HAMRS)	HAMRS helps to improve or promote intestinal function or environment.	2.5 g per serving	Nil
Lutein	Lutein as a predominant macular pigment in the retina that is able to filter blue light and helps to protect the eyes.	2.5 µg per 100ml (3.7 µg per 100 kcal)	This minimum level is specified for infant formula only.
		20 µg per 100ml (30 µg per 100 kcal)	This minimum level is specified for follow up formula only.
		20 µg per 100ml (20 µg per 100 kcal)	This minimum level is specified for formulated milk powder for children only.
Oligofructose/ fructo-oligosaccharide (FOS)	(i) FOS is a prebiotic. (ii) FOS is a bifidogenic. (iii) FOS helps to increase intestinal bifidobacteria and maintain a good intestinal environment.	1.25 g per serving	This minimum level is specified for food other than infant formula.
		0.4 g per 100 ml on a ready to drink basis	(i) This minimum level is specified for infant formula only. (ii) The component of inulin and FOS shall not exceed 0.6 g per 100 ml.
Polydextrose	(i) Polydextrose is a bifidogenic. (ii) Polydextrose helps increase intestinal bifidobacteria. (iii) Polydextrose helps maintain a good intestinal microflora.	1.25 g per serving	Nil
Soy protein	Soy protein helps to reduce cholesterol.	5 g per serving	There shall be written on the label the following statement: "Amount recommended to give the lowering effect on the blood cholesterol is 25 g per day".

Table 6. List of permitted other function claims and required conditions in Malaysia (cont'd)

Component	*Claims*	*Minimum amount required*	*Conditions*
Plant sterol or plant stanol or plant sterol ester	Plant sterol or plant stanol or plant sterol ester helps reduce cholesterol.	0.4 g per serving in a "free basis" form.	(i) Types of plant sterol or plant stanol permitted: "plant sterol or plant stanol, phytosterols or phytostanol, sitosterol, campesterol, stigmasterol or other related plant stanol". (ii) Types of plant sterol esters permitted: "campesterol ester, stigmasterol ester and beta-sitosterol ester" (iii) Amount of plant sterol or plant stanol or plant sterol ester in a "free basis" form to be added in food shall not exceed 3 g per day. (iv) Statement of the total amount of plant sterol or plant stanol or plant sterol ester contained in the product shall be expressed in metric units per 100 g or per 100 ml or per package if the package contains only a single portion and per serving as quantified on the label. (v) Only the terms "plant sterol" or "plant stanol" or "plant sterol ester" shall be used in stating the presence of such components. (vi) There shall be written on the label the following statements: a) "Not recommended for pregnant and lactating women, and young children under the age of five years"; b) "Persons on cholesterol-lowering medication shall seek medical advice before consuming this product"; c) "This product is consumed as part of a balanced and varied diet and shall include regular consumption of fruits and vegetables to help maintain the carotenoid level"; and d) "With added plant sterols or plant stanol or plant sterol ester" in not less than 10 point lettering".

Table 6. List of permitted other function claims and required conditions in Malaysia (cont'd)

Component	*Claims*	*Minimum amount required*	*Conditions*
Slowly digestible starch (SDS)	A food containing slowly digestible starch (SDS), consumed as part of the normal first meal of the day, releases carbohydrates gradually and provides energy throughout the morning	At least 40% of the available starch must be present as slowly disgestible starch (SDS)	Claim only permitted for SDS from starch naturally occurring in starchy foods where available carbohydrates provide at least 55 % of the total energy and where at least 55 % of the available carbohydrates is available starch.

For all the above claims, words/sentences of similar meaning can also be used
Source: Table IV of the Fifth A Schedule, Malaysia Food Regulations 1985 (MOH Malaysia, 2020)

discussion (for walk-in clients) and through results of their registration application.

However, industry may apply to the FDA for the use of any intended health claims related to bioactive non-nutritional food components. This is provided for under Administrative Order No. 2014-0029 Rules and Regulations on the Licensing of Food Establishments and Registration of Processed Food, and Other Food Products, and For Other Purposes (DOH Philippines, 2014). There is no specific prescribed form for submitting applications. No details of the requirements for application or type of scientific information required have been provided.

With regard to health claims for probiotics, the BFAD Circular in the Philippines has allowed the use of four pre-approved claims related to probiotics and can be reflected on the product labels, used for advertisement and product promotion (BFAD, 2004). These claims include enhancement of intestinal ecology, improvement of lactose malabsorption, improvement of digestion and aid to the enhancement of natural resistance to intestinal infections. Companies are permitted to apply for the use of these specified health claims on probiotics by going through the same review process as the procedure for evaluation of probiotics for food use.

Singapore

Health claims are permitted in Singapore, including nutrient function claims, other function claims and reduction of disease risk claims. A list of the three types of approved health claims is included in "A Guide to Food Labelling and Advertisements" (SFA, 2021). The definitions of these claims are as given in Codex Alimentarius Guidelines for Use of Nutrition and Health Claims (FAO/WHO, 2013). Other function claims describe the health effect of bioactive non-nutritional food constituents, e.g.

"Probiotics helps in digestion" or *"Inulin helps in calcium absorption"*. A total of 17 other function claims for 12 bioactive food components are in the list of accepted claims (SFA, 2021) (Table 7).

Industry may apply to the SFA for the use of new health claims, including other function claims using a prescribed application form. An outline of the application procedure is given in the Guide mentioned above (SFA, 2021). Besides the identity of the food constituent and its characteristics, reports of human intervention studies (at least 5 but not more than 10, and preferably published in the past 10 years) must be submitted to substantiate the proposed claim. Guidance information (including details or scientific data required), application form and checklist for application are provided in the document. Form for Application For Use Of Health Claims For Food Intended For Sale In Singapore is available from the SFA e-services website: https://www.sfa.gov.sg/e-services?type=food-manufacturing&page=1.

All applications will be reviewed by an expert committee which comprises reputable scientific experts with relevant professional training and experience from various government bodies, tertiary institutions and industry associations.

In the list of permitted other function claims mentioned above (Table 7), four function claims relating to the role of probiotics in helping to maintain a healthy digestive system through suppressing the growth of harmful bacteria may be used for probiotic-containing foods. The exact species of the probiotic present in the product must be specified on the label. The viable count of the probiotic present in the product that is able to bring about the claimed effect must also be indicated (SFA, 2021).

Thailand

Health claims are provided for under Food Act B.E. 2522, specifically sections 6(10), 40 and 41 (MoPH Thailand, 1979). A regulation on health claims is being drafted by the Thai FDA. In the meantime, details of the permitted health claims and the associated conditions of use are given in the Public Manual on Requesting for assessment of health claim by Thai FDA (FDA Thailand, 2020). Three types of health claims are permitted and their definition are as in the Codex guidelines on Nutrition and Health Claims (CAC/GL 23-1997), namely nutrient function claim, other function claim and reduction of disease risk claim. (FAO/WHO, 2013).

Other function claims are defined as *"presentations of properties or benefits that describe specific beneficial effects of the consumption of foods or their constituents, in the context of the total diet on normal functions or biological activities of the body; such claims relate to a positive contribution to health or to the improvement of a function or to modifying or preserving health"*.

The Public Manual outlines various criteria and conditions to be met for the health claims. Other function claims will not be permitted for foods that contain:

- Total fat more than 13 g, or
- Saturated fat more than 4 g, or
- Cholesterol more than 600 mg, or
- Sodium more than 360 mg

in amount of one recommended serving size and one serving size declared on labels, or if no recommended serving size specified, nutrient content in 100 g or 100 ml is calculated.

The Public Manual gives a great deal of emphasis on the importance of submitting the required scientific evidences to substantiate the applied claim. Health claims shall be based on relevant and available scientific evidence which is sufficient to prove relation between such claim and health. It consists of 2 parts of data including information on:

Table 7. List of acceptable other function claims in Singapore

Nutrients / Food constituents	*Claims*	*Criteria*
Chromium	Chromium contributes to normal macronutrient metabolism	• ≥ 6mcg in per 100g or 100ml of food • The amount of chromium must be declared under the nutrition information panel
Collagen	Collagen is a protein in connective tissues found in skin, bones and muscles	• The addition of collagen has to be disclosed under the statement of ingredients
Docosahexaenoic acid (DHA) and Arachidonic acid (ARA) – claim only for food for children up to 3 years of age	DHA and ARA are important building blocks for development of the brain and eyes for children up to 3 years of age.	• Food must be labelled clearly for this age group • The amounts of DHA and ARA must be declared under the nutrition information panel
Nucleotides - claim only for food for children up to 6 years of age	Nucleotides are essential to normal cell function and replication, which are important for the overall growth and development of children up to 6 years of age	• Food must be labelled clearly for this age group • The amounts of nucleotides must be declared under the nutrition information panel
Taurine - claim only for food for children up to 6 years of age	Taurine helps to support overall mental and physical development for children up to 6 years of age	• Food must be labelled clearly for this age group • The amount of taurine must be declared under the nutrition information panel
Inulin	1. Inulin helps in calcium absorption	• ≥ 133.33mg of calcium in per reference quantity of the food as specified Table 8 in section “Nutrition claims” • The amount of calcium must be declared under the nutrition information panel • The amount of inulin present in each serving or other equivalents of the product must be declared on the product label • Food manufacturer/importer to ensure that the amount and combinations of shorter and longer chain inulin present in the product can bring about the claimed effect.
	2. Inulin helps support growth or beneficial bacteria/good intestinal flora in gut	• Food manufacturer/importer to ensure that the amount of inulin present in the product can bring about the claimed effect.
	3. Inulin helps increase intestinal bifidobacteria and helps maintain a good intestinal environment	

Table 7. List of acceptable other function claims in Singapore (cont'd)

Nutrients / Food constituents	*Claims*	*Criteria*
Oligofructose (Fructo-oligosaccharides)	Oligofructose stimulates the bifidobacteria, resulting in a significant increase of the beneficial bifidobacteria in the intestinal tract. At the same time, the presence of less desirable bacteria is significantly reduced	• Food manufacturer/importer to ensure that the amount of the oligofructose present in the product can bring about the claimed effect.
Prebiotics	Prebiotic promotes the growth of good *Bifidus* bacteria to help maintain a healthy digestive system	• The exact identity of the prebiotic and must be declared on the product label • Food manufacturer/importer to ensure that the amount of prebiotic present in the product can bring about the claimed effect.
Prebiotic blend of Galacto-oligosaccharides and long chain Fructo-oligosaccharide	Prebiotic blend (galacto- oligosaccharides and long chain fructo- oligosaccharides) support the child's natural defenses for children up to 6 years of age	• The combination of Galacto- oligosaccharides and long chain Fructo-oligosaccharide present in the product must be in ratio of 9:1
Probiotics	1. Probiotics to help maintain a healthy digestive system 2. Probiotics helps in digestion 3. Probiotics helps to maintain a desirable balance of beneficial bacterial in the digestive system 4. Probiotics helps to suppress/fight against harmful bacteria in the digestive system, thereby helping to maintain a healthy digestive system	• The exact specie of the probiotic present in the product must be declared on the product label • Food manufacturer/importer • to ensure that the viable count of the probiotic present in the product can bring about the claimed effect.

Table 7. List of acceptable other function claims in Singapore (cont'd)

Nutrients / Food constituents	*Claims*	*Criteria*
Plant sterols/stanols	Plant sterols/stanols have been shown to lower/ reduce blood cholesterol. High blood cholesterol is a risk factor in the development of coronary heart disease	• Phytosterols, phytosterol esters, phytostanols or phytostanol esters may only be added to — (i) any edible vegetable fat or oil containing not more than 20 g of saturated fat per 100 g of total fat; (ii) any margarine or fat spread containing not more than 27 g of saturated fat per 100 g of total fat; or (iii) any other food containing not more than 3 g of total fat per 100 g or 1.5 g of total fat per 100 ml. • The following mandatory information must be declared on the product label: (i) The product is a special purpose food intended for people who want to lower their blood cholesterol level; (ii) The product may not be nutritionally appropriate for pregnant and breast- feeding women and children under the age of 5 years; (iii) The product should be used as part of a balanced and varied diet; (iv) Consumption in a day of a total of more than 3g of phytosterols and/or phytostanols does not provide any additional benefit in lowering blood cholesterol levels; (v) Consumption in a day of a total of at least 2g of phytosterols and/or phytostanols has been shown to lower blood cholesterol levels; and (vi) A statement suggesting the amount of the food (in g or ml) to be consumed each time (referred to as a serving), and a statement of the total amount of phytosterols and phytostanols that each serving contains.

Table 7. List of acceptable other function claims in Singapore (cont'd)

Nutrients / Food constituents	*Claims*	*Criteria*
Barley or Oat beta-glucan	Barley beta-glucans / Oat beta-glucans have been shown to lower/reduce blood cholesterol. High blood cholesterol is a risk factor in the development of coronary heart disease.	• The cholesterol, saturated fatty acids and trans fatty acids present in the food must be within the following levels: (i) in the case of solid food — a. not more than 20 mg of cholesterol per 100 g; b. not more than 1.5 g of saturated fatty acids and c. trans fatty acids per 100 g; and d. not more than 10% of kilocalories from e. saturated fatty acids and trans fatty acids; or (ii) in the case of liquid food — a. not more than 10 mg of cholesterol per 100 ml; b. not more than 0.75 g of saturated fatty acids and c. trans fatty acids per 100 ml; and d. not more than 10% of kilocalories from saturated fatty acids and trans fatty acids. • The following mandatory information must be declared on the product label: (i) a statement or statements to the like effect that consumption of at least 3 g of barley beta-glucans or oat beta-glucans (as the case may be) in a day has been shown to lower blood cholesterol levels; and (ii) the amounts of barley beta-glucan or oat beta-glucans (as the case may be), cholesterol, saturated fatty acids and trans fatty acids, present in the food under the nutrition information panel.

a. the physiological role of the nutrient or on an acceptable relationship between diet and health;
b. the composition of the product relevant to the physiological role of the nutrient or the acceptable relationship between diet and health unless the relationship is based on a whole food or foods whereby the research does not link to specific constituents of the food.

Applications for other function claims shall submit scientific evidence documents for consideration in the form of full copy of well-designed human intervention study published in reliable journal and either of the following document:

a. Systematic review or meta-analysis published in reliable journal or;
b. Recognised and reliable technical opinions from international recognised agencies, organisations, or scientific expert committee.

Supporting documents that may be submitted with the application include peer-reviewed published articles, animal study *in vivo, ex vivo*, or *in vitro*, observational evidence of epidemiological study which provide results consistent with the number of well-designed study, evidence-based reference texts, or other recognised and reliable texts (if any).

Details of the steps required to be taken by a corporate company when submitting an application for other function claim (or other health claims) is provided in detail in the afore-mentioned Public Manual. The review process undertaken by FDA is also outlined. Currently the Centre for Nutrition Assessment and Health Claims for Food Products of Thailand (CNACT) is designated by the Thai FDA for the review procedure. An ad hoc review/ expert committee involving a minimum of 3 experts from relevant subject areas relevant to the product/constituent is appointed to carry out the review. After completion of the assessment, a report is submitted to the Thai FDA for final approval. Once approved, the food component/constituent name and function can be displayed on food label. Advertisement can also be made after approval.

In relation to health claims for probiotics, a generic pre-approved health claim: *"Beneficial microorganism to the body"* or words of similar meaning may be used with prior approval by the Thai FDA (MoPH Thailand, 2011). Industry or importers may apply for probiotics health claims using a prescribed form. The regulation has provided clear guidelines for the criteria, procedure and conditions when submitting a health claim on probiotics. Scientific substantiation of the intended claim should be obtained from well-designed human intervention studies from at least two different institutes (MoPH Thailand, 2011).

Vietnam

There is no specific regulatory framework for making of health claims for biologically active ingredients in supplemented foods. The MOH Circular on Regulating Management of Functional Foods (MOH Vietnam, 2014) makes provision for the making of nutrition and health claims for functional foods. Article 8 outlines the health claims related to supplemented foods. The Circular emphasises that health claims can only be made when there is sufficient scientific evidence.

For probiotic-containing foods, there are no pre-approved health claims. However, companies may apply for health claims for these foods. The application for health claims follows the same procedure and requirements for application of a probiotic strain to be

registered for use under supplemented food or health supplement (Tee *et al.*, 2021).

DISCUSSION

From a review of the documents in Codex Alimentarius and the existing regulations in the eight SEA countries, it is clear that there is a great deal of importance being given to the role that bioactive food components may play in human health. These are not the traditionally recognised macro- and micro-nutrients.

There are significant differences in the regulatory control of the use of these bioactive food components in the eight countries in this review. Indonesia and Malaysia have promulgated specific regulations in relation to the addition of these bioactive non-nutritional food components in foods and beverages. Both countries have provided a positive list of the permitted bioactive food components that may be used, and also provided clear process for the industry to apply for additional such food components. Both countries have separate regulations that govern the making of function claims for non-nutrients, which refer to the beneficial physiological or health effects brought about by bioactive non-nutritional food components.

The other six countries in the review do not have specific regulations governing the use of bioactive non-nutritional food components. However, all these countries permit the making of other function claims, provided they are substantiated by scientific studies. This indicates the recognition given to the positive physiological roles that such non-nutritional components may have in the body.

All the eight countries in the review permit the making of function claims for non-nutritional substances. All these countries have regulations for this purpose or adopt Codex Alimentarius guideline on claims. Indonesia, Malaysia and Singapore have published positive list of permitted function claims for non-nutritional substances. Indonesia does not use the term “other function claim” for health benefits imparted by non-nutritional substances although the concept is the same.

All countries also permit industry to apply for additional function claims, though each country has slightly different requirements and process in place. One common requirement in all the countries is that applications must be accompanied by scientific evidence, preferably from human clinical trials.

In addition to the bioactive non-nutritional food components which as are the main focus of this review, all countries, except Myanmar allow the sale and marketing of probiotics in foods, another important functional food component. Indonesia, Malaysia, Philippines and Thailand have promulgated specific probiotic regulations and, except for Indonesia, have published positive list of probiotic strains that may be added to foods and beverages. All seven countries have provisions for the industry to apply for the use of probiotic strains, supported by the relevant documents on safety and beneficial effects. Malaysia, Philippines, Singapore and Thailand permit the use of a small number of pre-approved generic function claims related to probiotics.

CONCLUSION

Numerous epidemiological and clinical studies have demonstrated that bioactive non-nutritional food components or constituents are able to serve physiological roles beyond meeting basic nutritional requirements and may possess health-enhancing properties. This review has shown that all eight countries recognise the potential health

effects of these bioactive or functional components, and have made provisions in the regulatory framework for these non-nutritional substances. Two countries, Indonesia and Malaysia, have promulgated specific regulations to enable the addition of these components to foods with specific conditions. The other six countries, Brunei Darussalam, Myanmar, Philippines, Singapore, Thailand and Vietnam allow these food components to be used via applications for making other function claims, if they can be substantiated by scientific data. Codex Alimentarius guideline on claims has also a specific claim, other function claim, recognising the potential health benefits of such food components.

This review has indicated that there are significant differences in the approach to permitting the use of bioactive non-nutritional food components, the process for reviewing applications, as well as the associated other function claims. It does indicate that there are opportunities for sharing of experiences of the established systems, and discussion to potentially harmonise these processes among regulatory agencies in the region. Such a networking platform would be beneficial to the advancement of scientific and regulatory development of bioactive non-nutritional food components in the region and would benefit all stakeholders.

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Authors' contributions

Both authors contributed to the conceptualisation of paper, obtained regulations/documents for analysis, analysed and extracted relevant information, drafted manuscript, finalised manuscript for publication.

Conflict of interest

Authors declare that they have no conflicts of interest.

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